CHARACTERIZATION OF ABELSON MURINE LEUKEMIA VIRUS-TRANSFORMED MIDGESTATION EMBRYONIC CELLS AND THEIR NORMAL HOMOLOGUES

By

MICHAEL L. SIEGEL

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1986

ACKNOWLEDGEMENTS

This dissertation and my accomplishments over the past several years are the result of a team effort. I wish to acknowledge the contributions of all of my "teammates," and hope that I slight none by inadvertent oversight.

First, I would like to thank my friend, mentor, and supervisory chairperson, Edward J. Siden. His profound powers of perception and his ability to assimilate new observations into an existing body of knowledge have set ideals which I shall take with me. His friendship and camaraderie will always be of value to me.

Second, I wish to acknowledge and thank my supervisory committee, J. Bert Flanegan, Carlo Moscovici, Steve Russell, and Roy Weiner, who collectively guided me through my dissertation research, sometimes despite my reluctance and protestations.

My family has been a constant source of support, cheer, and inspiration. My wife Jeremie has coped with every crisis I brought home and has survived more mood fluctuations in the last few years than most people endure in a lifetime. David and Rebecca have also endured the journey with few complaints and good humor, and it is to them I owe the preservation of my humor. I must also thank my father, Seymour Siegel, who inspired me to strive to be the best at whatever I tried (even if I was a garbage man),

and my mother, Frances Helfer Siegel, who showed me the value of patience and determination. My brother, Victor, is also acknowledged for encouraging me to read and learn.

I owe much of my recent success to the support of my fellow graduate students. In particular, I express my thanks to Randy Horwitz, who has helped me stay young, sharpened my cynical wit, shared my most profane moments, and provided me with friendship which has endured almost six years in Gainesville.

Finally, I wish to recognize the faculty and staff (past and present) of the Department of Immunology and Medical Microbiology who have made my experience more fulfilling. In particular, I wish to thank Ken Berns, who encouraged me to return to graduate studies after a seven year hiatus. I am also indebted to Catherine and Richard Crandall, George Gifford, and Michael Boyle, for a seemingly endless supply of reagents and advice. Last, but not least, I thank Muriel Reddish, Patrice Boyd, Ellen Boukari and their superb staffs, without whom the work would probably have taken six additional years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CHARACTERIZATION OF ABELSON MURINE LEUKEMIA VIRUS-TRANSFORMED MIDGESTATION EMBRYONIC CELLS AND THEIR NORMAL HOMOLOGUES

By

Michael L. Siegel

May 1986

Chairman: Edward J. Siden
Major Department: Immunology and Medical Microbiology

The embryonic origin and ontogeny of mast cells is poorly understood, despite a growing body of literature relevant to that area of study. We have systematically investigated the development of mast cells in the embryonic mouse, beginning our studies with the observation of mast cell characteristics of midgestation embryonic placental cells transformed with the defective retrovirus, Abelson murine leukemia virus. Unlike previously reported Abelson virus-transformed cells, the placental cell lines exhibited many of the characteristics of culture-derived mast cells, including differentiation antigens, high affinity receptors for IgE, and metachromatic granules containing histamine and sulfated proteoglycans. Some of the cell lines also expressed the B220 marker previously reported to be specific for cells of the B lymphoid lineage. We also

developed a simple, sensitive, nonfluorometric, nonisotopic assay to detect membrane receptors for immunoglobulins.

The observation of the mast-like, Abelson virus-transformed cell lines led us to investigate the presence of mast cell precursors in normal midgestation embryonic tissues. We found embryonic precursors to mast cells in homologous, noninfected tissues and conducted a detailed, systematic analysis of the differentiation of mast cells in liquid cultures over the course of several weeks of selection and enrichment. We also studied the effects of Abelson virus infection and adherent cell cytokines on lymphoid differentiation antigens in mast cell cultures.

Mast cell precursors in embryonic tissues of mid- and late gestation were quantitated by a clonal assay. We described the embryologically earliest reported mast cell precursors in the mouse and report that the mouse embryo is a rich reservoir of such precursors, containing proportionately at least as many such cells as adult bone marrow. We have observed that mast cells which differentiate in agar culture, like some of the Abelson virus-transformed cell lines, express the B220 determinant. We have also described preliminary experiments in which we selected mast cell precursors in bone marrow on the basis of surface membrane determinants.

CHAPTER I
INTRODUCTION AND REVIEW OF THE LITERATURE

Introduction: Hematopoietic Cell Differentiation and Tumor Models

The ontogeny of the hematopoietic system of the mouse can be viewed as a progression of finite, genetically programmed stages in the maturation of pluripotent stem cells into the terminally differentiated state of each of the various blood lineages. Pluripotent hematopoietic stem cells, defined by their ability to reconstitute lethally irradiated recipients (Till and McCulloch, 1961), are first detected in the murine yolk sac between eight and twelve days of gestation (Tyan, 1968). Beginning with day ten and throughout the remainder of gestation, cells with the same differentiative capacity are found in the fetal blood and liver (Moore and Metcalf, 1970). In the adult, pluripotent hematopoietic stem cells are found in the bone marrow (Till and McCulloch, 1961) and spleen (Nakahata and Ogawa, 1982).

The mechanisms involved in the differentiation of pluripotent hematopoietic stem cells into mature, functional blood elements are, for the large part, unknown. These pathways may involve the interaction of pluripotent or committed progeny stem cells with other cells or macromolecular products in their inductive environment (Kincade et al., 1981a), resulting in the cell's commitment to one of several genetically programmed, phenotypically distinguishable chains of events; alternately, random

stochastic processes may play a role in the differentiation of hematopoietic cells (Nakahata et al., 1982a; Suda et al., 1984).

The differentiation pathways of hematopoietic cells and the molecular events involved in normal hematopoiesis are best understood and defined by delineating discrete cellular intermediates. Observation and identification is frequently hampered by hematopoietic tissue heterogeneity, short life span, and low frequency of cells of interest. These difficulties are overcome in part by virus-induced transformation of such cells, resulting in relatively homogeneous populations of adequate size and frequently unlimited growth potential. Although transformed homologues of normal cells are frozen at a particular stage of development by the action of the transforming gene product, virus infection may also induce phenotypic changes which are unparalleled in the course of normal differentiation. To avoid this pitfall, it is therefore prudent to confirm that the characteristics of tumor cell models of early blood progenitors mimic their naturally occurring counterparts.

Abelson murine leukemia virus (A-MuLV) is a replication-defective retrovirus which arose by recombination of portions of the genome of the replication-competent, thymotropic Moloney murine leukemia virus and a cellular gene, c-abl (Shields et al., 1979; Goff et al.,

1980) in a prednisolone-treated BALB/cCR mouse (Abelson and Rabstein, 1970). A-MuLV is capable of rapid transformation of bone marrow-derived thymus-independent lymphoid cells in vivo (Sklar et al., 1975; Premkumar et al., 1975) and in vitro (Rosenberg et al., 1975; Baltimore et al., 1979; Rosenberg and Baltimore, 1976a, 1980).

Although most of the in vivo transformants reportedly have been of the B cell phenotype (Premkumar et al., 1975), plasmacytomas have also been reported (Potter et al., 1973). T cell lymphomas (Cook, 1982), myelomonocytic leukemias (Raschke et al., 1978), and mast cell tumors (Mendoza and Metzger, 1976; Risser et al., 1978; Pierce et al., 1985) have also resulted from in vivo infections, indicating that Abelson virus may affect the growth and differentiation of multiple hematopoietic lineages.

Abelson murine leukemia virus is capable of transforming both hematopoietic and nonhematopoietic cells in vitro. Rosenberg and Baltimore (Rosenberg et al., 1975; Rosenberg and Baltimore, 1976a, 1976b) have developed an in vitro culture system for the transformation and clonal proliferation of lymphoid cells from murine hematopoietic tissues. Under well-defined conditions, permanent cell lines with pre-B lymphoid characteristics (Siden et al., 1979) have been generated. These cell lines are believed to exhibit the earliest known differentiation markers and immunoglobulin gene organizational structures

of pre-B cells (Boss et al., 1979; Siden et al., 1979; Alt et al., 1981, 1984). Using identical conditions for transformation of mouse placenta and fetal liver, Waneck and Rosenberg (1981) described colonies of cells expressing various differentiated erythroid characteristics, including cessation of growth. Unlike its Moloney leukemia virus ancestor, A-MuLV transforms the NIH/3T3 fibroblast cell line (Scher and Siegler, 1975).

Modifications of the original Rosenberg and Baltimore in vitro transformation protocol have resulted in the transformation of phenotypically disparate lineages. Whitlock and colleagues (1983) and Serunian and Rosenberg (1986) have reported the transformation of more differentiated B lineage cells from in vitro-derived "normal" populations. More recently, permanent cell lines expressing mast cell characteristics have been reported following transformation of midgestation embryonic placenta (Siegel et al., 1985) and third trimester fetal liver targets (Pierce et al., 1985).

Recent reports from several laboratories indicate that retroviruses may alter the growth factor requirement of cells of several lineages. Rapp and colleagues (1985) reported the development of factor-independent cell lines following transformation of interleukin 2-dependent T lineage and interleukin 3-dependent myeloid cell lines by recombinant viruses bearing the v-myc oncogene. Abelson

virus was similarly capable of abrogating the interleukin 3-dependence of both myeloid (Greenberger et al., 1979; Cook et al., 1985) and mast (Pierce et al., 1985; Chapter III, this dissertation) lineages. Taken with the observation of erythropoietin-independent erythroid cells (Waneck and Rosenberg, 1981) and interleukin 3-independent mast cells (Pierce et al., 1985; Siegel et al., 1985) from A-MuLV-infected primary cell populations, the data suggest that A-MuLV may alter the requirements of these cells for growth factors while permitting the cells to differentiate.

Although the precise role of the v-abl oncogene in maintaining factor-independent proliferation is not yet known, it is interesting to note that the cellular homologue, c-abl, is transcribed at its highest level in the developing embryo at the same time that the number of A-MuLV targets reaches its peak. At least two retroviral oncogene products have recently been shown to have analogous structures in normal cells. The epidermal growth factor receptor exhibits striking similarity to the erb B oncogene product of avian erythroblastosis virus (Downward et al., 1984), while the v-sis oncogene of simian sarcoma virus encodes a protein structurally and immunologically related to platelet-derived growth factor (Doolittle et al., 1983; Robbins et al., 1983; Waterfield et al., 1983). Although the function of c-abl has not been established, it is known that all of the hematopoietic lineages sensitive

to A-MuLV transformation are also sensitive to the proliferative effects of interleukin 3 (reviewed in Iscove and Roitsch, 1985; Rennick et al., 1985). Seminal studies on the culture of mast cells revealed that cells of this lineage would proliferate in the presence of media conditioned by lectin- or antigen-stimulated T lymphocytes or by the myelomonocytic leukemia cell line WEHI-3B (Hasthorpe, 1980; Nabel et al., 1981; Nagao et al., 1981; Schrader, 1981; Tertian et al., 1981). More recently, interleukin 3, the proliferative factor in the conditioned media, was purified to homogeneity (Ihle et al., 1983; Razin et al., 1984a). Subsequent studies performed with the glycoprotein product of cloned interleukin 3 gene have substantiated the proliferative activity of the factor (Yokota et al., 1984; Rennick et al., 1985).

Mast Cell Function, Origin, Ontogeny, and Heterogeneity

Contemporary knowledge and interest in the mast cell has its roots in the midnineteenth century. The earliest description of these cells is found in the work of von Recklinghausen (1863), who observed and illustrated the mast cell in the unstained mesentery of the frog. Credit for the discovery of the mast cell, however, is generally assigned to Paul Ehrlich, a young physician-scientist who was then interested in the differential staining affinities of certain tissue cells and their organelles. Ehrlich (1877) first described mast cell-like cells in several

species as elements which stained atypically red-violet with the blue basic analine dye dahlia. The term mast cell is derived from Ehrlich's nomenclature (Mastzellen), which he assigned to granular cells which were abundant in well-nourished tissues of frogs (Ehrlich, 1879). In the same work, Ehrlich first used the term "Metachromasie," or metachromasia, to describe the anomalous staining of these connective tissue cells. Aside from their role as histochemical curiosities, however, few references to mast cell derivation, functions, and heterogeneity were published in the ensuing fifty years (Selye, 1965).

Mature mast cells have been attributed with a number of physiologic functions, many of which have been reviewed in the recent literature (Austen, 1984; Shanahan et al., 1984; Katz et al., 1985a; Lagunoff, 1985). The most widely known mast cell function is the anaphylactic response, which was first described by Selye in 1937 (cited in Selye, 1965). The response was originally experimentally induced in rats by intraperitoneal injection of egg white and produced hyperemia and edema of the lips, ears, paws, and genitalia which were aggravated by adrenalectomy and ablated by stressors like formaldehyde, which induce adrenocortical hyperplasia. The release of histamine during anaphylactoid inflammation was hypothesized at this time, although release of the mediator was not experimentally associated with mast cells until 1954

(Benditt et al., 1954). Other substances shown to be released during the anaphylactic response include heparin and related proteoglycans, leukotrienes, prostaglandins, 5-hydroxytryptamine and other amines, and neutral proteases (reviewed in detail in Austen, 1984).

Mast cells have been directly or indirectly implicated in a number of other physiologic roles. Histamine has been associated with modulation and regulation of the immune system (Askenase et al., 1981) including reduction of T cell effector function (Plaut et al., 1973; Schwartz et al., 1980) and decreased lymphokine production (Rocklin, 1976). Mast cells have also been implicated in delayed-type hypersensitivity reactions (Askenase, 1977), immune complex formation (Benveniste et al., 1972), natural cytotoxicity (Farram and Nelson, 1980; Henderson et al., 1981), and parasite resistance (Capron et al., 1978), as well as elaboration of a factor akin to interleukin 1 which potentiates inflammation and collagenase activity in synovial cells (Van Den Hoof and Tichelar-Guttar, 1983; Yoffee et al., 1984, 1985).

Nonimmune functions have also been ascribed to mast cells. "Microenvironmental hormones" (Lewis and Austen, 1981) produced by mast cells have been implicated in tissue growth and repair (Kahlson and Rosengren, 1968) and thyroxine secretion by the thyroid (Melander, 1977). Histamine was shown to be required for timely blastocyst

implantation (Nalbandov, 1971; Dey and Johnson, 1980a, 1980b; Dey, 1981), as were prostaglandins (Kennedy, 1977), but the number of detectable mast cells in the gravid uterus was shown to decrease after implantation (Shelesnyak, 1960; Brandon and Bibby, 1979). Mast cell association with nerve tissue was first noted in 1878 (cited in Selye, 1965) and intimate contact between nerve endings and mast cell plasma membranes was documented by Weisner-Menzel and colleagues (1981) and Newson and colleagues (1983). Histamine release has been attributed to stimulation of cutaneous nerves (Kiernan, 1972), thus establishing a neuroendocrine-mast cell axis. Mast cells in alimentary tract mucosa have been implicated in promotion of gut mobility (Erjavek et al., 1981; Fjellner and Hagermark, 1981); histamine-induced gastric secretion was shown to be blocked by histamine H2 receptor agonists (Soll et al., 1981) and enhanced by glucocorticoids (Sathiamoorthy et al., 1976). The latter observation is contradictory to published reports of glucocorticoid-induced suppression of intestinal anaphylaxis (King et al., 1985), and may indicate different modes of action of steroids on sensitized and nonsensitized mast cells. Thus, Daeron and colleagues (1982) noted that glucocorticoids inhibited antigen-induced, but not calcium ionophore A23187-induced, histamine release from mast cells.

The derivation of mast cells in mammalian tissues has been the subject of considerable investigation, and sometimes heated debate, since the early nineteen sixties. Because the developmentally earliest described mast cells were observed in connective tissue, and a gradation of "immature" to "mature" forms of mast cells could be isolated from this source, several investigators proposed that mast cells were derived directly from connective tissue precursors (Burton, 1963; Michels, 1963). Combs and colleagues (1965) observed the development of mast cells in embryonic rats between fifteen and twenty-three days post coitum. The cells appeared to arise in undifferentiated mesenchymal tissue and progressed through a gradation of intermediates to characteristic mast cells. Asboe-Hanson (1971) further noted that mast cells in the skin appeared to differentiate locally from mesenchymal elements.

Based on observations of profound mastocytosis associated with immune and neoplastic lymphocyte proliferation, a second faction proposed the hematopoietic origin of mast cells. Accordingly, Ginsburg (1963) removed thymuses from mice and cultured them with embryonic skin monolayers. The mast cells so derived led the author (Ginsburg, 1963; Ginsburg and Lagunoff, 1967) to propose that mast cells were derived from thymocytes, an observation subsequently confirmed in the rat (Ishizaka et al., 1976). Burnet (1965, 1975, 1977), observing that mast

cells exhibited evidence of thymic origin or dependence, participated in immunologic reactions, were similarly activated by lectins and immune stimuli, and were relatively amitotic, speculated that the mast cells were "post-mitotic" T lineage cells.

Other hematopoietic cell types have been postulated to be mast cell by precursors in vivo and in vitro cultivation techniques. Desaga and colleagues (1971) performed repeated peritoneal lavages on rats to deplete them of mature serosal mast cells. Mast cell-deficient peritoneal exudate cells, cytochemically identified as monocytes at the beginning of in vitro growth, developed into metachromatic, granulated mast cells within two days of harvest. Similarly, Czarnetzki and Behrendt (1981) reported that peritoneal exudate cells from mast cell depleted rats (injected with sterile water intraperitoneally) resembled mononuclear phagocytes both morphologically and cytochemically before and shortly after culture in L-cell conditioned media. The in vitro-propagated population, however, differentiated into mast cells which were identified morphologically and which contained granules with histamine and alpha-naphthol acetate esterase.

The hematopoietic origin of some, and perhaps all, mast cells was defined in great detail by Kitamura and colleagues in a series of reports beginning less than ten

years ago. Initially, they demonstrated that irradiated, mast cell-deficient mice could be reconstituted by injection of bone marrow from untreated donors (Kitamura et al., 1977). Since irradiation did not eradicate all recipient mast cells prior to reconstitution, a donor strain with phenotypically distinct mast cell granules (beige) was used to definitively demonstrate the origin of the cells which were detected. Similar experiments, using unirradiated, genetically mast cell-deficient W/W^V mice, demonstrated that adult bone marrow (Kitamura et al., 1978; Hatanaka et al., 1979), blood (Kitamura et al., 1979a) and spleen (Kitamura et al., 1979d) were rich reservoirs of mast cell precursors which were also found in smaller numbers in thymus, lymph node, and Peyer's patches (Kitamura et al., 1979d). Mast cells were also detected in fetal liver populations despite the apparent lack of mature mast cells in that tissue (Kitamura et al., 1979c).

The hematopoietic nature of the in vivo mast cell precursor was further defined by the same group. In preliminary studies, Kitamura and colleagues (1981) showed that genetically mast cell-deficient W/W^V mice could be reconstituted with cells from individual spleen colonies of normal (C57BL/6) mice which had been irradiated and subsequently reconstituted with bone marrow cells. Having assured themselves of the clonality of each donor spleen colony (by injecting mixed phenotypically distinct beige

and wild type bone marrow cells into the primary, irradiated recipients and screening the colonies for cells with only one type of granule), the authors were able to conclude that the colony forming unit of the spleen (Till and McCulloch, 1961) was the ultimate progenitor cell for mast cells in the spleen, stomach, caecum, and skin as well as for peripheral blood granulocytes and erythrocytes. These results were corroborated by Sonada and colleagues (1983), who demonstrated that late (twelve days post injection) spleen colonies, which included both erythroid and myeloid elements, contained mast cell precursors which differentiated in secondary recipient skin.

The ontogeny of embryonic mast cells in mice is meagerly defined in the literature. As previously mentioned, in vivo mast cell precursors are present, despite the absence of more mature forms, in the mouse fetal liver thirteen and more days post coitum (Kitamura et al., 1979c). Embryonic mast cell ontogeny was better defined in the rat. Csaba and Kapa (1960) demonstrated the presence of mast cells, which incorporated exogenous heparin, in the thymus, spleen, lymph nodes, myocardium, and kidney of day sixteen to seventeen rat embryos. By studying sections through rat embryos between fifteen and twenty-three days of gestation, Combs and colleagues (1965) placed mast cells into four stages of differentiation based upon their staining characteristics with alcian blue and

safranin, dyes which preferentially bind to the glycosaminoglycan components of mast cell granules. By this technique, alcian blue binds to poorly sulfated glycosaminoglycans like chondroitin sulfate, while safranin binds to highly sulfated molecules like heparin. A gradation of cells, beginning with large, lymphocyte-like elements with few, alcian blue-stained granules (Stage I), and progressing through characteristic mature mast cells with small nuclei and large numbers of safranin-staining granules (Stage IV), was documented. Mast cell stages were also differentiated by nuclear characteristics (mitotic figures), granule heparin (periodic acid-Schiff staining), granule glycosaminoglycan synthesis (sodium sulfate uptake), granule histamine (diazotized parabromoaniline reaction), and granule protease (phenylproprionyl naphthol AS reaction) content. The first recognizable mast cells (Stage I) were found in the head mesenchyme at fifteen days of gestation. Mast cell numbers rapidly increased during the sixteenth day of gestation, with Stage II mast cells found in the connective tissue of the dorsal vertebrae. Subcutaneous (Stage III) mast cells were identified in tissues of embryos eighteen and nineteen days post coitum. Mature Stage IV mast cells were first present shortly before birth and were indistinguishable from adult connective tissue mast cells.

The study of mast cells, which has accelerated dramatically in the past quarter century, has revealed that Ehrlich's "Mastzellen" are heterogeneous both evolutionarily (interspecies) and functionally (intraspecies). Mast cell heterogeneity has been the topic of numerous general reviews in recent years (Enerback,1981; Bienenstock et al., 1982, 1983; Pearce, 1982, 1983; Shanahan et al., 1984; Jarrett and Haig, 1984; Austen, 1984: Katz et al., 1985a; Lagunoff, 1985; Pearce et al., 1985). From this sea of literature, the differences between mast cells of various species are apparent. The multipotent biogenic compound of mammalian mast cells, histamine, has not been detectable in fishes and amphibians, while serotonin is apparently unique to rodent and dopamine to bovine mast cells. Heparin proteoglycan from porcine, bovine, rat, and human sources is heterogeneous in molecular weight and charge (Stevens and Austen, 1981). The proteolytic enzymes of mast cells are also phylogenetically disparate; rodent mast cells contain an alpha-chymotrypsin-like activity, while dog, human, and turtle mast cells have trypsin-like activity and bird and fish mast cells have no esteroprotease activity (Woodbury and Neurath, 1980; Lagunoff, 1985). Ultrastructurally, the granules of human mast cells appear to be organized in crystalline scrolls, while rat mast cell granules are

dense, homogeneous spheres which dissociate into fibrillar structures in hypertonic salt solution (Lagunoff, 1972).

The initial observation of intraspecies mast cell heterogeneity is generally attributed to Maximow (1906), who reported that the rat intestine was replete with mast cells which differed from other rat mast cells in morphology and stain affinity. These differences were reinvestigated by Enerback, who, sixty years after Maximow's observations, published a series of reports which described in detail the differences in morphology, stain affinities (Enerback, 1966a, 1966b), and sensitivity to degranulating agents (Enerback, 1966c, 1966d) between dermal mast cells (representative of the connective tissue or serosal subset) and intestinal mast cells (representative of mucosal or atypical mast cells). Thus, mucosal mast cells were shown to be smaller, possess uni- or bilobed nuclei, and be less granulated than serosal mast cells, and the granules of the former population were far more heterogeneous in size than those of the latter. Enerback also observed that the mucosal mast cells stained red with acidic toluidine blue, while serosal mast cells stained purple. He noted that standard formaldehyde fixatives used to preserve serosal mast cell granules were ineffective on mucosal mast cells, and selected and adapted several fixatives (such as Carnoy's and Mota's preparations) to more adequately preserve the more labile

mucosal mast cell granules. Mucosal mast cells also required higher concentrations of thiazine dyes, like toluidine blue, and azure A dyes, as well as prolonged staining times, when compared to serosal mast cells, while the granules of the former cells had higher affinity to copper phthalocyanine dyes such as Astra blue at ph 0.3. Enerback therefore concluded that the mucosal mast and connective tissue mast cells differed not only morphologically, but biochemically as well, and offered that the mucosal mast cells contained less highly sulfated mucopolysaccharides than the dermal cells. Finally, Enerback noted that in rats systemically exposed to the histamine releasing agent 48/80, serosal mast cells in the mesentery, tongue, and skin were degranulated and therefore undetectable, while mast cells in the duodenal mucosa were unaffected and perhaps greater in number. While being unable to explain the latter observation, Enerback was able to conclude that mucosal mast cells differed from their serosal counterparts functionally as well.

The differences between mucosal and serosal mast cells, first noted by Maximow and then Enerback, have since been appended by the observations of numerous investigators and extend beyond those mentioned to surface markers, histamine content, IgE receptors and internalization of bound IgE, proteoglycan composition, proteases, sensitivity to histamine secretagogues, effects

of neuropeptides and endorphins, thymus dependence, and life span. These characteristics have been surveyed in detail in reviews previously cited. We will therefore only briefly survey the literature which is cogent to the ultimate topic of this discussion, the in vitro, culture-derived mast cell.

Connective Tissue Mast Cells

Although typical mast cells have been isolated from a variety of connective tissue sources throughout the rodent body, the most frequently studied member of this subset is that which is isolated, free of extraneous tissues, from the serosal surfaces of the peritoneal cavity. As previously discussed, by injecting bone marrow into mast cell-deficient hosts, Kitamura and colleagues (1977) were able to demonstrate the relationship of hematopoietic precursors to the serosal mast cells. The ultimate precursor cell in the bone marrow was shown to be the colony-forming unit of the spleen (Kitamura et al., 1981; Sonoda et al., 1983). From the bone marrow, mast cell precursors migrate through the blood (Kitamura et al., 1979a; Zucker-Franklin et al., 1981; Sonoda et al., 1983) and subsequently proliferate and differentiate in connective tissue (Hatanaka et al., 1979; Kitamura et al., 1979b,1979d). Connective tissue mast cells in rodents have been found to proliferate and differentiate independently of thymic influences. Thus, the athymic nude mouse has mast

cells in its connective tissues (Wlodarski, 1976; Reed et al., 1982). Aldenborg and Enerback (1985) recently reported that congenitally athymic rnu/rnu rats have at least as many (or more) peritoneal mast cells as normal controls for the first fourteen weeks of life; adult rnu/rnu rats, however, have fewer peritoneal mast cells than their wild type counterparts. These results may indicate that peritoneal mast cell populations may be subject to thymic influences later in life or may simply reflect a separate, thymus-independent defect inherent in the athymic rat. Further studies will be necessary to elucidate the apparent contradiction.

The proteoglycan composition of serosal mast cell granules has been the subject of study since the initial discovery of heparin in the canine liver by Jorpes in 1937 (cited in Selye, 1965). Subsequently, heparin has been identified in rat peritoneal mast cells (Tas and Berndsen, 1977; Yurt et al., 1977; Stevens and Austen, 1982), human lung mast cells (Metcalfe et al., 1979), and mouse peritoneal mast cells (Razin et al., 1982c).

Mucosal Mast Cells

Following the development of improved methods for their fixation and staining by Enerback (1966a, 1966b), the study of the atypical, or mucosal, mast cell accelerated significantly. Early reports of similarities between cultured, thymus-derived mast cells of the mouse (Ginsburg,

1963; Ginsburg and Lagunoff, 1967) and the rat (Ishizaka et al., 1976) and Enerback's atypical mast cells of the lamina propria spurred erroneous theories that the mucosal mast cell was derived from the thymus (Burnet, 1965, 1975, 1977). Based upon observations of mucosal mastocytosis following experimental infection of rats and mice with the nematodes Nippostrongylus brasiliensis and Trichinella spiralis, a lymphoid origin of these cells was also proposed by other investigators (Rose et al., 1976; Befus and Bienenstock, 1979; Mayrhofer, 1979a,1979b; Nawa and Miller, 1979).

The derivation of mucosal mast cells was ultimately resolved by Crowle (1982), who reconstituted mucosal mast cell-deficient mice with cells derived from a variety of hematopoietic tissues. Crowle observed that W/W^V mice could be reconstituted by bone marrow and spleen cells but not by thymocytes or thymus grafts, while athymic mice could be reconstituted by thymus grafts, thymocytes or splenocytes. Crowle proposed that the W/W^V mice were defective in mucosal mast cell precursors which were present in bone marrow (and spleen) of normal mice, while athymic mice possessed the precursor population and needed a thymus-related component to effect differentiation. Crowle concluded that mucosal mast cells were derived from bone marrow and required a thymic influence for accumulation in mucosal surfaces. These relationships

were further reinforced by the same investigator (Crowle and Reed, 1984). Reconstitution of athymic mice was ablated by pretreatment of wild type mouse bone marrow cells or splenocytes with anti-Thy 1 and complement, while similar treatment of beige bone marrow or spleen cells still resulted in the detection of some mast cells, albeit fewer, in the mucosa of thymus-intact W/W^V mice.

The thymic dependence of mucosal mast cells, in contrast to the thymus-independent growth and development of serosal mast cells, has been documented by a number of other investigators. Prior to the reports of Crowle (1982,1984), Ruitenberg and Elgersma (1976) observed that nude mice infected with Trichinella spiralis experienced no intestinal mucosal mast cell response unless reconstituted by thymus or parasite-immune thoracic duct cell grafts, concluding that thymus-derived T-lineage cells were required for mucosal mastocytosis. These results were substantiated by a number of other researchers (Olson and Levy, 1976; Mayrhofer and Bazin, 1981; Reed et al., 1982). Similar studies were performed in the rat. Mayrhofer (1979a) noted that the number of mucosal mast cells in Nippostrongylus brasiliensis-infected rats increased in a pattern similar to primary and secondary immune responses. Adult thymectomy or chronic thoracic duct drainage several months prior to nematode challenge (to deplete mature T cells) resulted in significantly depressed intestinal mastocytosis, while

thymectomy shortly before challenge was ineffective in ablating the mast cell response (Mayrhofer, 1979b). These last observations indicated that the thymus, per se, is not the immediate source of mast cells or mast cell growth factors. Similar depression of mucosal mast cell response and poor clearance of intestinal parasites were observed in B rats, thymectomized, irradiated animals which were reconstituted with bone marrow of T cell-depleted (chronic thoracic duct drainage) donors (Mayrhofer and Fisher, 1979). Interestingly, contrary to the reports of Crowle (Crowle, 1982; Crowle and Reed, 1984), unchallenged B rats, as well as athymic nu/nu mice, were reported to have normal numbers of mucosal mast cells when compared to appropriate controls (Mayrhofer and Bazin, 1981).

Three other independent lines of evidence fortified the hypothesis that mucosal mast cells were dependent on a T cell-derived proliferation-differentiation factor. First, the primary mucosal mast cell response to Nippostrongylus brasiliensis in the rat was enhanced by adoptive transfer of immune T cells (Nawa and Miller, 1979). Second, mucosal mastocytosis was demonstrated in a variety of other immune scenarios, including the inflammatory reactions of ulcerative colitis, Crohn's disease, and pulmonary fibrosis (Askenase, 1980). Third, Guy-Grand and colleagues (1984) recently reported the direct stimulation of intestinal mucosal mast cell

precursors in BALB/c mice bearing the myelomonocytic leukemia WEHI-3, a constitutive producer of the mast cell growth factor interleukin 3, which is identical to the mast cell growth factor produced by activated T lymphocytes (Yung et al., 1981).

A variety of other distinguishing characteristics have been ascribed to the cells alternately called mucosal mast cells, atypical mast cells, and histaminocytes (Code, 1977). Early studies of mucosal mast cells were performed on tissue sections or on heterogeneous populations of cells isolated from the gut mucosa. The previously cited methodologies of Enerback (1966a, 1966b) were later optimized by the inclusion of techniques which further stabilized the granules (neutral formalin fixation) and enzymatically (with trypsin) stripped stain-retarding proteins from glutaraldehyde-treated preparations (Wingren and Enerback, 1983). The recent development of methods for isolating such cells from the small intestine, which exploited the mucosal mastocytosis induced by parasitic infection, was reported by Befus and colleagues (1982a), and made it possible to analyze mucosal mast cells in the absence of extraneous elements and to confirm some previous observations. In contrast to peritoneal mast cells, isolated mucosal mast cells are smaller and have a shorter lifespan. Mucosal mast cells have fewer granules which contain nonheparin, lower sulfated proteoglycans (as yet

biochemically undefined), less histamine, and serotonin (Bienenstock et al., 1983).

The response of mucosal mast cells to a variety of secretagogues has been the subject of a number of studies. Similar to serosal mast cells, mucosal mast cells are responsive to the degranulation effects of IgE and antigen, IgE and anti-IgE, concanavalin A, ionomycin, and compounds 23187 and Br-X537A, albeit with the release of less of their total histamine content (Befus et al., 1982a, 1982b; Pearce et al., 1982). Enerback's early observations on the insensitivity of rat mucosal mast cells to degranulation by compound 48/80 in vivo were confirmed by other investigators in canine (Lorenz et al., 1969; Rees et al., 1981) and murine (Enerback, 1981) models. This unresponsiveness was confirmed in isolated mucosal mast cells and extended to the secretagogue Bee Venom Peptide 401 (Befus et al., 1982a, 1982b), for which membrane receptors were found to be absent on mucosal, but not serosal, mast cells (Pearce et al., 1982). The same group also reported that, unlike serosal mast cells, mucosal mast cells were unresponsive to enhanced, antigen-induced secretion of histamine mediated by phosphatidyl serine. Mucosal mast cells were also shown to be distinct from serosal mast cells in their responsiveness to secretory antagonists disodium chromoglycate, theophylline, and AH9679, while both subsets were equally sensitive to the

degranulation inhibitory effects of Doxantrazole (Befus et al., 1982b; Pearce et al., 1982, 1985).

Basophils

Cells with membrane receptors for IgE are not limited to the mast cell lineage. Both lymphocytes (Gonzales-Molina and Spiegelberg, 1978) and macrophages (Melewicz et al., 1982) have been reported to bind IgE; the affinity of the membrane receptors of the non-mast cells, however, was ten to one-hundred times less than that of mast cells (Ogawa et al., 1983). The best known of the mast-like cells, however, are the basophils (basophilic granulocytes), polymorphonuclear leukocytes which are present in the blood of several mammalian species (Lagunoff and Chi, 1980). Like mast cells, basophils contain metachromatic granules and express surface membrane receptors for IgE. Histamine is released by antigenic challenge of IgE-bearing basophils (Lagunoff and Chi, 1980). The granules of basophils of several species have been reported to contain chondroitin sulfate proteoglycan (Olsson et al., 1970; Orenstein et al., 1978; Metcalfe et al., 1980b) similar in its degree of sulfation to the proteoglycan of mucosal mast cells (Tas and Berndsen, 1977). Basophilic granulocytes, however, are apparently absent from mouse peripheral blood (Lagunoff and Chi, 1980). The relationship of these cells to culture-derived

mast cells in several species, including the mouse, will be further discussed in a later section of this review.

Mastocytomas

The study of mastocytomas has contributed to the understanding of mast cell growth, differentiation, and function, and has provided the bridge between complex in vivo investigations and better-defined, clonal population analyses of in vitro cultures. Spontaneous mastocytoma, while common in such species as dogs (Cobb et al., 1975; Yoffee et al., 1984, 1985), is a more infrequent condition in other species, notably rats and mice (Lagunoff, 1985). Efrati and colleagues (1957) described human mastocytoma cells as large, lymphocyte-like elements similar to the early mast cell progenitors described in lower mammals (Maximow, 1906).

The link between in vivo and in vitro mast cell studies was established in 1959 when Schindler and colleagues (1959) reported the successful adaptation of the methylcholanthrene-induced, murine mastocytoma P815 (Dunn and Potter, 1957) to growth in culture. The latter investigators had isolated the neoplasm from a disseminated disease with foci in the spleen and subcutaneous tissue and had subsequently adapted it to a highly transplantable ascites form from which a number of observations were made. Variations in cell morphology, granule size and density, and nuclear morphology were noted in the descriptive study.

In vitro analysis (Schindler et al., 1959) demonstrated that the cells synthesized histamine and serotonin. Through extensive culture, some sublines of P815 lost both granules and intracellular histamine content; in some sublines, however, the condition was reversed by addition of sodium butyrate to the culture medium (Mori et al., 1979).

Focal mastocytomas have frequently been associated with the presence of lymphocytes (Galli and Dvorak, 1979; Askenase, 1980), supporting the once popular hypothesis that the latter cells were precursors to the former (Burnett, 1965, 1975, 1977). It was also noted that some mastocytomas, like basophils and culture-derived mast cells, synthesize chondroitin sulfate proteoglycan rather than the heparin (Lewis et al., 1973) found in serosal mast cells.

Some mastocytomas have been reported during the course of experiments analyzing Abelson murine leukemia virus-induced lymphomagenesis (Mendoza and Metzger, 1976; Risser et al., 1978). The mastocytomas generally arose in peritoneal oil granulomas evoked by tetramethylpentadecane (Pristane) in mice which were inoculated with Abelson virus thirty to forty days after administration of the oil (Pierce et al., 1985). The tumors were generally transplantable into syngeneic hosts and frequently could be adapted to growth in vitro. In a yet unexplained, but

perhaps related note, Hasthorpe (1980) reported the isolation and adaptation to in vitro growth of a factor-dependent mast-like cell line from splenocytes of a DBA-2 mouse infected with Friend erythroleukemia virus. Although electron microscopy established the presence of budding C-type virus particles on the highly granular cells, Hasthorpe's FMP1.1 cell line was nontumorigenic upon intraperitoneal or subcutaneous administration to syngeneic hosts and could not proliferate in the absence of exogenous growth factor in vitro.

Culture-Derived Mast Cells

The growing body of knowledge concerning mast cells is due, in part, to the development of methodologies for the selection, enrichment, and maintenance of culture-derived mast cells. These cells possess many of the characteristics of their in vivo correlates, the mucosal mast cells, as demonstrated in the following text. A note of caution, however, must be interjected into the seemingly logical flow between naturally occurring in vivo mast cells and culture-derived mast cells. Although similar by a variety of criteria including morphology, biochemistry, function, and growth factor dependence, culture-derived mast cells are not "normal" in the sense that they have been produced in anatomically foreign, although perhaps physiologically sufficient, conditions. They may therefore

be considered models of their in vivo homologues until definitive evidence permits us to conclude that the two populations are completely identical. We will therefore continue to use the term "culture-derived" mast cells, and similarly distinctive terms, to maintain the tenor of this caveat throughout the following discussion.

Adherent Feeder Layer Studies

The earliest reports of culture-derived mast cells by Ginsburg (Ginsburg,1963; Ginsburg and Sachs, 1963) involved a complex system of mouse thymocytes cultured on feeder layers of mouse embryonic fibroblasts. It was apparent to the authors that the monolayer was essential for proliferation of mast cells. Culture of thymocytes in the absence of the feeder layer failed to produce mast cells. On the other hand, culture of embryonic skin fibroblasts from eighteen day fetuses, without additional thymocytes, infrequently resulted in mast cell outgrowth. The tissue source of the mast cells, therefore, was disputable, and quite possibly both thymocytes and embryonic feeder layers contributed progenitors to the mast cell culture. The issue was better defined several years later when the same group reported that irradiated embryonic fibroblast monolayers, which could no longer produce mast cells when

cultured alone, could support the growth of culture-derived mast cells from cocultured thymocytes (Ginsburg and Lagunoff, 1967).

Thus, the culture-derived mast cell era was ushered in with the observation that at least some mast cell progenitors were present in lymphoid tissue and that adherent cells were required for in vitro mast cell differentiation and growth. In seminal attempts to clone mast cell precursors in soft agar, Pluznik and Sachs (1965) reported that embryonic feeder layers were again required for outgrowth of mast cells from disaggregated splenocytes. Ishizaka and colleagues (1977), investigating rat thymus-derived mast cells in a system modeled after that of Ginsburg, observed that clonal expansion was more prolific in the presence of a feeder layer, although mast cells were, indeed, isolated from cultures containing only thymocytes. In the latter case, however, the mast cells were observed to be associated with islands of fibroblast-like adherent cells which arose in the thymocyte cultures.

The origin of mast cells in feeder layer cultures was better resolved, almost twenty years after its initial identification, by the definition of two morphologically distinct populations of mast cells in mixed cultures of adult lymphoid and embryonic feeder layer cells (Ginsburg et al., 1982). Mast cells derived from the feeder layer were morphologically similar to those found in connective

and serosal tissues, while those of lymphoid origin (lymph node and thoracic duct) resembled mucosal mast cells, being smaller in size with sparser, but larger granules than the former cells. Pure "mucosal mast cells" (in fact, culture-derived mast cells of lymphoid origin) could be grown on selected feeder layers which were free of serosal type precursors. The "mucosal mast cells" persisted, however, only in the presence of T cell-derived factors. In contrast, the mast cells derived from embryonic feeder layers continued to persist, albeit without further expansion, for six months or longer in the absence of exogenous factors.

The same group (Davidson et al., 1983) later reported that lymph node cells derived from unimmunized, horse serum-immunized, and helminth-infected mice, grown in the presence of conditioned media (from antigen-stimulated mesenteric lymph node cells) but in the absence of irradiated embryonic mouse fibroblast feeder layers, proliferated (as large, vacuolated cells) but failed to develop granules. When the undifferentiated, culture-derived cells were transferred to fibroblast monolayers, however, the cells developed metachromatic granules containing histamine within seven days. Intimate contact between the two populations of viable cells was apparently essential to granule maturation, as neither fibroblast conditioned media, fibroblast homogenates, glutaraldehyde-

fixed fibroblasts, nor separation of fibroblasts from "large lymphocyte" mast cell precursors by a membrane could effect the change.

Despite the initial success in culturing mast cells from lymphoid tissue cocultivated with adherent cell feeder layers, reports in the literature of the technique's use were limited to those of the previously cited groups. Two factors probably contributed to the limited use of adherent cell monolayers in mast cell culture. First, the system was quite complex, requiring considerable time and extensive subculture (or irradiation) of feeder layers to eliminate the contribution of connective tissue type mast cell precursors. Secondly, and perhaps more significantly, the development of culture-derived mast cells in media conditioned by activated lymphocytes by at least three independent groups provided the opportunity to maintain mast cells in the absence of a continuous monolayer of feeder cells. As previously noted, however, even in the absence of fibroblast feeder layers, islands of adherent cells are observed in early cultures of lymphoid and hematopoietic tissue-derived mast cells (confirmed in our studies; see Chapters III and IV). Since the adherent cells are present in such cultures before the selection and enrichment of mast cells, it is, at this juncture, plausible to speculate that the adherent cells may assume a transient, maturational role in mast cell differentiation.

Conditioned Media-Dependent Mast Cells

The development of techniques for culturing mast cells from hematopoietic and lymphoid tissues has led to an exponential increase in mast cell research and literature citations. With the burst of scientific activity, however, has come a concurrent increase in the number of terms used to describe culture-derived mast cells, including P (persisting) cells (Schrader and Nossal, 1980), histamine-containing granular cells (Sredni et al., 1983), mucosal mast cells, basophil/mast cells, and atypical mast cells. Despite the discrepancy of terms, however, the long term suspension cultures of mast cells appear to be strikingly similar. Mast cells have thus been derived from mouse bone marrow (Tertian et al., 1980, 1981; Nagao et al., 1981; Razin et al., 1981a, 1982a,b,c; Schrader, 1981; Schrader et al., 1981; Galli et al., 1982b; Crapper and Schrader, 1983; Sredni et al., 1983; Wedling et al., 1983, 1985; Yung et al., 1983; Suda et al., 1985), spleen (Hasthorpe, 1980; Schrader and Nossal, 1980; Schrader et al., 1980, 1981; Schrader, 1981; Tertian et al., 1981; Crapper and Schrader, 1983; Sredni et al., 1983; Pharr et al., 1984), fetal liver (Nabel et al., 1981; Razin et al., 1984b), peripheral blood (Crapper and Schrader, 1983; Suda et al., 1985), thymus (Tertian et al., 1981; Schrader, 1981; Davidson et al., 1983), lymph nodes (Ginsburg et al., 1978; Crapper and Schrader, 1983), and intestinal mucosa (Schrader et al.,

1983b). Similar cells have been cultured from rat bone marrow (Haig et al., 1982, 1983), peripheral blood (Zucker-Franklin et al., 1981; Czarnetzki et al., 1983), and thymus (Ishizaka et al., 1976, 1977) as well as human fetal liver (Razin et al., 1981b), umbilical cord blood (Ogawa et al., 1983), and adult peripheral blood (Denburg et al., 1983; Czarnetzki et al., 1984).

The development of in vitro methods for mast cell culture provided additional means of detecting embryonic mast cell precursors. As previously noted, Ginsburg (1963) observed occasional mast cell outgrowth in cultures of day eighteen embryonic mouse skin. Similar experiments with the rat model demonstrated that embryonic rat thymus, isolated between eighteen and twenty days post coitum and cocultured with adult rat thymocytes or thymocyte conditioned media, contained cells capable of differentiating into mast cells (Ishizaka et al., 1976). A third group (Nabel et al., 1981; Galli et al., 1982a) was able to culture murine mast cells derived from day thirteen fetal liver suspensions cultured in lymphocyte conditioned media. Using the adherent cell system, Ginsburg and colleagues (1982) were also able to demonstrate that mast cells could be derived in culture from disaggregated mouse embryos between ten and thirteen days of gestation. It was thus apparent that the precursors of culture-derived mast cells were present in the mouse embryo at ten days post

coitem, several days before mast cells per se were observable in the embryo (Kitamura et al., 1979c).

Interleukin 3 From Lymphocyte-Conditioned Media And Other Sources

The analysis of culture-derived mast cells and their precursors evolved from studies of hematopoietic cell growth factors produced by lymphocytes. Reports of mast cell-supporting factors (which, for the sake of convention, we will commonly call interleukin 3) in the supernatants of mitogen-stimulated splenocytes began to surface at the beginning of the present decade (Burgess et al., 1980; Hasthorpe, 1980). Since that time, a number of other investigators have utilized media conditioned by a variety of means to support the differentiation and growth of culture-derived mast cells. Such media have thus been derived from splenocytes activated by concanavalin A (Clark-Lewis and Schrader, 1981; Tertian et al., 1981; Yung et al., 1981; Schrader et al., 1981; Nakahata et al., 1982b; Yung and Moore, 1982; Sredni et al., 1983), by pokeweed mitogen (Hasthorpe, 1980; Nakahata et al., 1982b, Wedling et al., 1983, 1984; Pharr et al., 1984), by phytohemagglutinin A (Ogawa et al., 1983), by bacterial lipopolysaccharide (Nakahata et al., 1982b), and by mixed lymphocyte reactions augmented by lectin (Razin et al., 1981a, 1982a, 1982c). Conditioned media with analogous activity have been elicited from concanavalin A-stimulated

mesenteric lymph node cells of parasitized animals (McMenamin et al., 1985). Phytohemagglutinin A- or concanavalin A-stimulated human blood lymphocytes also produced activities which supported the growth of human basophilic cells and a growth factor-dependent mouse cell line (Tadokoro et al., 1983; Stadler et al., 1985).

The phenotype of the murine lymphoid cell which produces interleukin 3 was deduced from the activities of conditioned media of a number of related T cell clones. In contrast to Lyt 1+2+, Lyt 1-2+, and Lyt 1-2- cells, which did not support culture-derived mast cell growth, the supernatants of Lyt 1+2- T cell clones, corresponding to the inducer T lymphocyte subset, supported the proliferation of such cells (Nabel et al., 1981). The observations of Nabel and colleagues were subsequently confirmed by other investigators (Fung et al., 1984; Yokota et al., 1984), and by the observation that rat mesenteric lymph node cells expressing differentiation markers of helper T lymphocytes (OX19+, W3/25+, OX8-) were responsible for the production of a factor with analogous activity to mouse interleukin 3 (McMenamin et al., 1985).

A number of permanent cell lines also produce factors which support the growth of culture-derived mast cells. The best known of these cell lines is the myelomonocytic WEHI-3 line, which constitutively produces high levels of interleukin 3 (Nagao et al., 1981; Schrader et al., 1981;

Yung et al., 1981; Yung and Moore, 1982). Mast cell-promoting activities have also been demonstrated in conditioned media from concanavalin A-stimulated T cell hybridoma cells (Clark-Lewis and Schrader, 1981), cloned T cell lines (Nabel et al., 1981), lectin-stimulated T leukemias (Yung et al., 1981; Yung and Moore, 1982; Metcalf and Kelso, 1985), and B lymphoma cells (Clark-Lewis et al., 1982).

The biologically active factor in lymphocyte and WEHI-3 conditioned media has been given a variety of names. It was first termed "multi-CSF" by Burgess and colleagues (1980), due to its ability to support the differentiation of multiple hematopoietic lineages in vitro. Schrader and colleagues (Schrader and Nossal, 1980; Schrader, 1981; Schrader et al., 1981; Clark-Lewis and Schrader, 1981) reported that "P cell stimulating factor" (PSF), which supported the growth of persisting, mast-like cells in vitro, was present in concanavalin A spleen conditioned media. Ihle and colleagues (1981, 1982) proposed the name "interleukin 3", or IL 3, for the factor which induced the enzyme 20-alpha-hydroxysteroid dehydrogenase in nude mouse spleen cells as well as effecting a number of differentiative and supportive activities in multiple cell lineages. Although Ihle's terminology has achieved the greatest usage in recent literature, alternative nomenclature for the same activity has been proposed by

Bazill and colleagues ("multi-hematopoietic cell growth factor", MCGF; Bazill et al., 1983) and Iscove ("multilineage hematopoietic growth factor", multi-HGF; Iscove, 1985).

Interleukin 3, derived from a variety of sources, has been purified to homogeneity (Yung and Moore, 1982; Ihle et al., 1982b; Bazill et al., 1983; Clark-Lewis et al., 1984) and, more recently, the genes for IL 3 have been molecularly cloned and expressed (Fung et al., 1984; Yokota et al., 1984; Rennick et al., 1985). Extensive reviews of the literature describing interleukin 3 (per se, and the related activities called by various other names) have been published recently and should be consulted for additional information (Clark-Lewis et al., 1985; Ihle, 1985; Iscove, 1985; Schrader et al., 1985; Whetton et al., 1985; Yung and Moore, 1985).

Interleukin 3-Independent Mast Cells

Several reports of interleukin 3-independent mast cells have appeared in the literature in recent years. Schrader's group (Schrader and Crapper, 1983; Schrader et al., 1983a) observed the emergence of factor-independent variants from factor-dependent cells. In one experiment, factor-dependent cells were plated in agar in the absence of exogenous interleukin 3. From these cultures, several colonies of autonomous culture-derived mast cells (P cells) arose which, after several weeks, were subsequently adapted

to growth in interleukin 3-free liquid media. The autonomous colonies secreted interleukin 3 into the culture media, but also retained their receptors for the factor. Furthermore, the autonomous cells generated more colonies when plated at low density in the presence of exogenous interleukin 3 in agar than in the absence of the growth factor. It seems likely that the "autogenous" cells were not truly factor-independent, but rather were variants which were able to proliferate, albeit at lower efficiency, at the low levels of interleukin 3 provided by an autocrine mechanism.

Similar factor-independent culture-derived mast cells have been reported by a second group (Ball et al., 1983; Conscience and Fisher, 1985). Several long-term bone marrow-derived cultured mast cell lines were found, after eleven months in culture, to contain variants which proliferated at higher rates than similar cultures in the presence of interleukin 3. The more proliferative cells were able to continue cell growth in the absence of exogenous interleukin 3, but the doubling time was increased 160 percent when compared to cells of the same line maintained in conditioned media. In contrast to the autogenous cells of Schrader, the media conditioned by the factor-independent mast cells described in the more recent studies failed to support the growth of other factor-dependent culture-derived mast cells. It is possible,

however, that the cells were able to maintain their growth in the presence of interleukin 3 at levels below those detected in the assay. Interestingly, both sets of factor-independent mast cells were tumorigenic in syngeneic mice. Although no retroviral particles were observed in one of these cell lines (Ball et al., 1983), the observations are, by the criteria of tumorigenicity and factor independence, similar to those of a recent report of Abelson murine leukemia virus-transformation of culture-derived mast cells (Pierce et al., 1985). Two possible mechanisms could reconcile the yet unexplained results. First, the activation of a latent replication-defective viral genome in the long-term culture-derived mast cells of Ball and colleagues could be the missing link. Under such circumstances, no viral particles would be detected, but the cells could become both factor independent and tumorigenic by virtue of the viral transforming gene product. Similarly, the activation of a cellular homologue of a viral transforming gene, like c-abl (the normal function of which is unknown), could be invoked to activate the mechanisms necessary to generate the phenotype of the factor-independent, tumorigenic mast cells.

Mast Cell Precursors

The development of *in vitro* techniques for the differentiation and maturation of mast cells from phenotypically immature progenitors permitted the analysis

of mast cells based upon a number of criteria. First, the number of culture-derived mast cell precursors in particular tissues was determined quantitatively. Prior to the development of mast cell culture techniques which utilized sources of interleukin 3 for mast cell proliferation, Pluznik and Sachs (1965) enumerated mast cell clones in soft agar with feeder layers. The authors reported approximately thirty mast cell colony forming units per million spleen cells seeded, while the frequency of culture-derived mast cell precursors in embryos, thymus, and lymph nodes were five per million, three per hundred million, and less than one per fifty million cells, respectively. Schrader and colleagues (1981) cloned mast cell precursors from mouse bone marrow in soft agar with WEHI-3 conditioned media and found thirty to two-hundred progenitors per million Thy 1-negative cells. These results were contradicted by a more recent report (Sredni et al., 1983) which found 600 to 700 bone marrow precursors, 400 to 500 spleen precursors, 25 to 30 thymus precursors, and 100 to 200 lymph node precursors per million seeded cells. The latter results, however, were generated in a system using concanavalin A-activated splenocyte conditioned media as a source of interleukin 3 and different strains of mice, thereby making comparison difficult. Interestingly, the latter authors also observed that athymic nude mice had as many mast cell precursors as

syngeneic wild type controls. Since athymic mice do not exhibit the profound mucosal mastocytosis found in appropriate controls (Olson and Levy, 1976), the last observations could be interpreted to indicate that athymic mice lack the inductive conditions required for the proliferation of seemingly normal numbers of mast cell precursors.

Mast cell precursors were enumerated by Nakahata and colleagues (1982), using a modification of the semisolid methylcellulose media culture system previously used to identify erythroid and myeloid precursors. In the latter and subsequent report using this system (Pharr et al., 1984), which used pokeweed mitogen activated spleen conditioned media for a source of interleukin 3, the investigators found between twenty and 140 mast cell precursors per million BDF1 mouse spleen cells and 200 mast cell precursors per million bone marrow cells. Suda and colleagues (1985), using the Nakahata culture system, demonstrated that W/W^V mice, which are severly deficient in mast cells of both serosal and mucosal subsets, had the same number of peripheral blood mast cell precursors as wild type mice (approximately thirty per million nucleated cells), thus indicating that the mast cell defect was in a homing or developmental step, rather than at the stem cell or migratory level.

A third method for the enumeration of culture-derived

mast cell precursors from various tissues was reported by Crapper and Schrader (1983). Using limiting dilution analysis of cells in liquid culture containing WEHI-3 conditioned media, The authors were able to enumerate mast cell precursors in bone marrow, spleen, mononuclear peripheral blood cells, and lymph nodes. All of the data recorded in the latter experiments concurred with the previously cited results of Schrader and colleagues (1981) as well as those reported by the Nakahata group (Nakahata et al, 1982b; Pharr et al., 1984). Furthermore, Crapper and Schrader were able to substantiate the findings of Suda and colleagues that mast cell deficient mice had similar numbers of culture-derived mast cell precursors (in bone marrow and spleen) when compared to appropriate wild-type controls, although the former authors used W^f/W^f and the latter authors used W/W^v.

The development of techniques for the propagation of culture-derived mast cells has also permitted the characterization of such cells at various stages of differentiation. Thus, Ginsburg and colleagues (Ginsburg, 1963; Ginsburg and Sachs, 1963; Ginsburg and Lagunoff, 1967; Ginsburg et al., 1982; Davidson et al., 1983) reported a progression of characteristics of cultured mast cells, starting with large, mononuclear "stem" cells. After six to ten days in culture, large, lymphocyte-like "mastoblasts" with round and bilobed nuclei and a narrow

rim of metachromatic cytoplasm, similar to those described by Maximow (1906) in stained tissue sections, arose and became dominant in cell culture. The cytoplasm of the monoblasts continued to increase in size as the nucleus became more distinctly indented, with a chromophobic region in the concave aspect of the nucleus. At twelve to thirteen days in culture, a foamy region in the cytoplasm was seen to spread, sometimes encompassing the entire cytoplasm. Metachromatic material first appeared in the foamy region as faint, amorphous substance in vacuoles, later increasing in size and staining intensity to well-defined granules. These latter cells were described as "young mast cells", possessing granular, metachromatic cytoplasm, with actively mitotic, kidney-shaped, round or oval nuclei; such cells dominated the cultures between days twelve and twenty-two. After three weeks in culture, the majority of the cells were mature, round mast cells with round to oval, eccentric or centered amitotic nuclei and abundant, metachromatic cytoplasmic granules, similar in morphology and histochemistry to mucosal mast cells. Similar staging of rat culture-derived mast cell precursors and intermediates has been reported (Ishizaka et al., 1976; Zucker-Franklin et al., 1981; Sterry and Czarnetzki, 1982; Czarnetzki et al, 1983), corroborating in situ observations (Combs et al., 1965).

Characteristics of Culture-Derived Mast Cells

The development of refined analytical methods, first applied to other hematopoietic cells, aided in the characterization of culture-derived mast cells. Yung and colleagues (1983) analyzed bone marrow cells by centrifugation techniques and reported that interleukin 3-responsive cells could be isolated by nature of their median buoyant density (1.033) from interleukin 2-responsive cells (1.075). The authors also noted that the buoyant densities of long-term bone marrow-derived cultured mast cells (1.062 to 1.095 g/ml) were similar to those determined by Pretlow and Cassidy (1970), who analyzed heterogeneous populations of freshly isolated peritoneal mast cells and reported that immature mast cells have a median buoyant density of 1.087. Interleukin 3-responsive cells were separated by sedimentation velocity analysis from the in vitro precursors of macrophages (CFU-M) and the pluripotent colony-forming cell of the spleen (CFU-S), but not the bipotent precursor of granulocytes and macrophages (CFU-GM).

Culture-derived mast cells and their precursors have been characterized for the expression of a broad variety of hematopoietic differentiation markers in attempts to assign them to a particular lineage. Bone marrow and intestinal precursors to culture-derived mast cells were observed to lack the T lineage antigens Thy 1, Lyt 1 and Lyt 2

(Schrader et al., 1983b; Guy-Grand et al., 1984). Like their precursors, more differentiated culture-derived mast cells are deficient in Thy 1, Lyt 1, and Lyt 2 (Ginsburg et al., 1981; Nabel et al., 1981; Schrader, 1981; Schrader et al., 1981; Tertian et al., 1981; Davidson et al., 1983; Sredni et al., 1983; Ghiara et al., 1985), although Schrader and colleagues (1982) reported that Thy 1 may be transiently expressed on these cells. Similarly, culture-derived mast cells lack surface immunoglobulin, a B lineage marker (Ginsburg et al., 1981; Schrader et al., 1981; Tertian et al., 1981; Sredni et al., 1983), NK-1, a marker of natural killer cells (Nabel et al., 1981), complement receptors and MAC-1, a differentiation antigen of mononuclear phagocytes which is also expressed by some natural killer and T lymphoma cells (Tertian et al., 1981).

Like their in vivo correlates, murine culture-derived mast cells express surface receptors for IgE (Ginsburg et al., 1978; Nagao et al., 1981; Schrader, 1981; Schrader et al., 1981; Tertian et al., 1981; Ginsburg et al., 1982; Nakahata et al., 1982b; Sredni et al., 1983; Wedling et al., 1983, 1985), which induce the anaphylactic release of histamine when cross-linked by IgE and homologous antigen (Ginsburg et al., 1978; Sredni et al., 1983) or anti-IgE (Ginsburg et al., 1982). The number of IgE receptors per cell has been estimated to be 2 to 3 x 10^5, similar to the number on serosal and mucosal mast cells (Razin et al.,

1981a; Ginsburg et al., 1982). Some investigators have also noted the presence of receptors for IgG (Schrader, 1981; Tertian et al., 1981); the cells, however, did not phagocytose opsonized or unopsonized targets (Schrader, 1981; Sredni et al., 1983). Liquid cultured mast cells in conconavalin A-stimulated spleen conditioned media also express the lymphocyte marker Ly 5 (Nabel et al., 1981; Tertian et al., 1981). Contradictory observations of histocompatability Class II (Ia) antigens, or the lack thereof, on culture-derived mast cells were resolved by Wong and colleagues (1982), who showed that such cells, grown in the presence of immune interferon (interferon gammma, found in the supernatants of concanavalin A-stimulated splenocytes) expressed the marker while cells grown in the absence of interferon (as in WEHI-3 conditioned media) were devoid of Ia. Culture-derived mast cells were also shown to express Class I histocompatability antigens and receptors for peanut agglutinin (Schrader, 1981; Schrader et al., 1981; Tertian et al., 1981).

Russell and colleagues developed a panel of rat monoclonal antibodies against murine mononuclear phagocytes which could discriminate between culture-derived mast cells and connective tissue mast cells (Leblanc et al., 1982). The same group observed that culture-derived mast cells expressed the phenotype B1.1-/B23.1+/ B54.2+, while in contrast peritoneal mast cells were B1.1+/ B23.1-/B54.2+

(Katz et al., 1983). Although the Forsmann glycolipid recognized by monoclonal antibody B1.1 is undetectable on culture-derived mast cells, the latter cells do express the antigen precursor, globotetrasylceramide (Katz et al., 1985b), and may therefore be deficient or defective in the glycosyltransferase required for the synthesis of the mature antigen.

Culture-derived mast cells have been extensively characterized biochemically as well. The histamine content of cultured mast cells, like that of mucosal mast cells, has been estimated between 450 and 500 nanograms per million cells, at least ten-fold less than the histamine content of comparable numbers of peritoneal mast cells (Nabel et al., 1981; Nagao et al., 1981; Razin et al., 1981a; Galli et al., 1982b; Sredni et al., 1983; Wedling et al., 1985). Mouse culture-derived mast cell (and mucosal mast cell) granules stain blue when treated with alcian blue and safranin (Ginsburg and Lagunoff, 1967), indicating the presence of weakly sulfated mucopolysaccharides, whereas serosal mast cell granules, containing strongly sulfated heparin proteoglycan, stain red. Razin and colleagues (1982c) analyzed the proteoglycan of murine culture-derived mast cells and found they contained glucuronic acid-N-acetylgalactosamine-4,6-disulfate, or chondroitin sulfate proteoglycan E, a unique glycosaminoglycan which could not be detected in basophilic

leukemia cells, peritoneal mast cells, or chondrocytes. Chondroitin sulfate proteoglycan E was shown in this study to be chemically distinct from heparin by a number of criteria including sensitivity to enzymatic degradation and molecular weight (chondroitin sulfate proteoglycan E has an estimated molecular weight of 200 kilodaltons, in contrast to heparin, which has a molecular weight of 750 kilodaltons). These results have been confirmed in the literature (Razin et al., 1983; Sredni et al., 1983). Mouse culture-derived mast cell granules also contain a number of other in vivo mast cell-associated biological mediators, including serotonin (5-hydroxytryptamine) and dopamine (Tertian et al., 1981).

Arachidonic acid metabolites, the prostaglandins and leukotrienes, are important biological mediators asssociated with metachromatic cells. IgE-dependent activation of mouse culture-derived mast cells results in the synthesis and release of leukotriene C4 (Razin et al., 1982b, 1983), a component of the slow releasing substance of anaphylaxis (Austin, 1984). These studies also showed that bone marrow-derived cultured mast cells generated approximately twenty-five times more leukotriene C4 than prostaglandin D2 upon activation by calcium ionophore A23187 or IgE receptor-mediated pathways. In contrast, rat

peritoneal mast cells preferentially synthesized and released prostaglandin D2 in forty-fold excess over leukotriene C4.

Culture-derived mast cells are ultrastructurally distinct from serosal mast cells. Cells of the former category thus possess granules which are more heterogeneous in size and electron density than the latter (Ginsburg and Luganoff, 1967; Ginsburg et al., 1978; Nabel et al., 1981; Galli et al., 1982b; Wedling et al., 1985). Granules in culture-derived mast cells are ovoid and are frequently associated with small vessicles (Sredni et al., 1983) and a well-developed Golgi apparatus (Ginsburg and Lagunoff, 1967). The substance of mouse culture-derived mast cell granules is crystalline (Razin et al., 1982a), while that of human origin (resembling basophils, rather than mast cells) is more particulate (Razin et al., 1981b). Mouse bone marrow-derived mast cells form membrane channels after activation with IgE and anti-IgE through which granules may reach the cell surface (Razin et al., 1982a), similar to human lung mast cells (Caulfield et al., 1980). The cytoplasmic membrane of culture-derived mast cells is characterized by numerous, fine protrusions (Galli et al., 1982b; Wedling et al, 1985) which are absent from peritoneal mast cell membranes.

The response of culture-derived mast cells to secretagogues is similar to that of mucosal mast cells, but

not serosal mast cells. As previously discussed, leukotriene C4 is synthesized and released as a result of activation of the IgE receptor-mediated pathway. In addition, histamine, chondroitin sulfate proteoglycan E, and beta-hexosaminadase are released by immune activation (Razin et al., 1983). Like both serosal and mucosal mast cells, culture-derived mast cells are induced to degranulate by the calcium ionophore A23187 (Razin et al., 1982a, 1982b; Sredni et al., 1983; Robin et al., 1985). Culture derived mast cells, however, mimic mucosal mast cells in their lack of response to compound 48/80 (Sredni et al, 1983). In contrast, mouse peritoneal mast cells are degranulated by compound 48/80.

Many of the characteristics previously described for mouse culture-derived mast cells have been reported in analogous rat and human systems. Ishizaka and colleagues (1976) cultured rat thymocytes in the presence of rat embryonic fibroblast monolayers and observed the outgrowth of cells with receptors for IgE and metachromatic granules. Haig and colleagues (1982, 1983) grew rat bone marrow-derived cultured mast cells in the presence of media conditioned by mesenteric lymph node cells. The investigators observed that, similar to culture-derived murine mast cells, rat culture-derived mast cells were smaller than peritoneal mast cells, possessed sparse granules of heterogeneous size, and expressed surface

receptors for IgE. Rat culture-derived mast cell granules stained blue by the alcian blue-safranin technique and were metachromatic when stained with toluidine blue. The granule proteoglycan was identified as non-heparin, although no report of its precise chemical composition has been published to date. Such cells also contained immunochemically detectable levels of rat mast cell protease II, which was previously described as a marker of mucosal, but not serosal mast cells. Rat mesenteric lymph nodes (Denburg et al., 1980) and peripheral blood (Zucker-Franklin et al., 1981) have also been shown to contain precursors of culture-derived mast cells.

Reports of mast cells derived from human tissues are clouded by difficulties in distinguishing between the various types of basophilic cells when compared to in vivo correlates, namely mucosal mast cells, connective tissue mast cells, and basophils. Granulated cells with receptors for IgE and low levels of histamine (50 to 450 nanograms per million cells), thus resembling mouse culture-derived mast cells, have been observed in cultures of human fetal liver grown in unconditioned media (Razin et al., 1981b). Adherent human blood mononuclear cells and pleural exudate cells, which were propagated with L-cell conditioned media, exhibited similar characteristics (Czarnetzki et al., 1983, 1984; Kruger et al., 1983). Cells with high affinity receptors for IgE and slightly higher levels of histamine

(480 to 1600 nanograms per million cells) were cultured from human umbilical cord blood grown in phytohemagglutinin A-stimulated human T cell conditioned media (Ogawa et al., 1983). Horton and O'Brien (1983) reported the culture of granulated cells with centrally placed, round or indented nuclei from human bone marrow harvested from a patient with systemic mastocytosis. The culture-derived mast cells showed no growth advantage with a number of conditioned media, but required an adherent, bone marrow-derived feeder layer to persist.

Other In Vitro-Derived Metachromatic Cells

As noted previously, some of the confusion in the terminology applied to culture-derived mast cells has been generated by the appearance of characteristics of one or more of the *in vivo* basophilic cell correlates. Thus, although basophils in mice are lacking (Lagunoff and Chi, 1980) or extremely rare (Dvorak et al., 1982), a report of cloned, basophil-like cell lines with IgE receptors has appeared in the literature (Galli et al., 1982a). The cell line in question, derived from mouse splenocytes cultured in concanavalin A-stimulated splenocyte conditioned media, lacked histamine and had both natural killer cell differentiation markers and natural killer activity.

In a series of studies on the rat, Czarnetzki and colleagues described the growth of connective tissue-like mast cells *in vitro*. In the earliest study (Czarnetzki et

al., 1979), mast cell-free peritoneal exudate cells were harvested from rats which were previously injected with sterile water (intraperitoneally). The peritoneal cells were cultured in L-cell conditioned media with sodium butyrate. The mast cells which grew out of this population, although initially possessing blue-staining granules by the alcian blue-safranin technique, later had red staining granules and released histamine in response to compound 48/80. Despite their serosal mast cell characteristics, these culture-derived mast cells were similar to the classical description of mucosal mast cells in that they contained low levels of histamine (500 nanograms per million cells) and survived for only short periods. Similarly described cells were subsequently isolated by the same group from rat peritoneal cells (Czarnetzki and Behrendt, 1981), and rat mononuclear phagocytes (Czarnetzki et al., 1981, 1982; Sterry and Czarnetzki, 1982).

In related studies, in vitro cultivated human peripheral blood mononuclear cells (Denburg et al., 1983) and guinea pig bone marrow cells (Denburg et al., 1980) developed into metachromatic cells with segmented nuclei which were more characteristic of basophils than mast cells. The human cells, in particular, possessed the polymorphonuclear structure with mature chromatin, Golgi and microtubules which are more characteristic of basophils

than mast cells. This conclusion, however, directly disagreed with Zucker-Franklin (1980), who contended that human mast cells and basophils share common ultrastrucural organization.

Tadokoro and colleagues (1983) cultured cells with metachromatic granules and lobulated nuclei from normal human bone marrow in conditioned media from lectin-stimulated blood lymphocytes. The culture-derived cells contained 500 to 2000 nanograms of histamine per million cells and were responsive to IgE-anti-IgE- and calcium ionophore-mediated histamine release but were refractory to the effects of compound 48/80. The authors concluded that their conditioned media contained a basophil-promoting activity which furthermore had a molecular weight of 25 to 40 kilodaltons and was distinct from interleukin 2. The same group recently reported that media conditioned by phytohemagglutinin A- and concanavalin A-stimulated human blood lymphocytes could support the interleukin 3-dependent mouse cell line 32Dcl as well as promote the growth of human culture-derived basophils (Stadler et al., 1985). Furthermore, the interleukin 3 and basophil-promoting activities, which were also found in media conditioned by the growth of E-rosetting T lymphocytes and the MoT cell line, were biochemically distinct by at least five different criteria. The isolated human interleukin 3 was shown to promote the growth of mast cells which were unable

to proliferate in the presence of mouse interleukin 3 (in WEHI-3 conditioned media). Thus, in the human system, the culture-derived mast cell-basophil dilemma is no longer solely a matter of terminology and mistaken identity, but, in fact, apppears to involve multiple growth promoters and, most likely, multiple progenitor cells.

Relationship of In Vivo- and In Vitro-Derived Mast Cells

A small body of evidence supports the theory that culture-derived mast cells are more than circumstantially related to mucosal, and perhaps serosal, mast cells. A number of characteristics, including morphology, histochemical fixation and staining, dependence of proliferation on T cell-derived factors, biogenic amine content, protease content, presence of receptors for IgE, and sensitivity to secretogogues, have been noted in this review and cited by many of the authors as proof of the relationship between culture-derived mast cells and the mast cells of the mucosal surfaces. There is preliminary evidence that culture-derived mast cells have natural cytotoxic activity against tumors such as WEHI-3 and Meth A which is enhanced by interleukin 3 (Ghiara et al., 1985). Investigators have noted that in vivo-derived mast cells exhibited similar tumoricidal activity (Farram and Nelson, 1980) and that in vivo-derived cells with the

Thy 1-/Lyt 1-/ Lyt 2- phenotype, which are demonstrably cytotoxic, are sensitive to the proliferative activities of interleukin 3 (Djeu et al., 1983; Lattime et al., 1983).

Evidence of more direct relationships between in vitro-derived mast cells and their in vivo correlates has been elusive. Several investigators have associated the high incidence of culture-derived mast cell precursors and mucosal mast cells in the intestine of normal mice (Crapper and Schrader, 1983; Guy-Grand et al., 1984). The role of antigenic stimulation and T cell function in the proliferation of mucosal mast cells per se has been thoroughly described in the literature (for reviews see Jarrett and Haig, 1984; Shanahan et al., 1984; Bienenstock et al., 1983). Guy-Grand and colleagues (1984) also showed that the number of mast cells which could be cultured from intestinal mucosa increased with antigenic stimulation and WEHI-3 tumor burden, implicating the role of interleukin 3 in the in vivo proliferation of mast cell precursors. The results, however, associated the in vivo and in vitro mast cell precursors by existence in the same tissue, and did not directly show that the populations involved were identical.

The most suggestive evidence to date of the relationship between in vivo- and in vitro-derived mast cells involves the demonstration that culture-derived mast cells, when injected into mast cell-deficient mice,

populated both mucosal and connective tissue-serosal compartments. Nakano and colleagues (1985) injected culture-derived mast cells and partially purified (30 to 40 percent) peritoneal mast cells into W/W^v mice by various routes. At high levels of inoculum (10^5 to 10^6 cells), intravenously- or intraperitoneally-injected cultured mast cells populated the spleen and stomach (mucosa and muscle), while at lower levels of inoculum (10^2 to 10^4cells), only intraperitoneally injected cells were able to populate the same anatomical sites. Both cultured and peritoneal mast cells were relatively inefficient at populating the skin, however, possibly due to the presence of mast cells, or their precursors, which were already present in the skin (Kitamura et al., 1977). An interesting note to the Nakano studies was the evolution of mucosal or serosal mast cell characteristics from injected cells (regardless of origin) depending on the anatomical site of subsequent lodging. Thus, the granules of mast cells isolated from the peritoneal cavity, spleen, skin, and gastric muscularis propria of reconstituted animals stained preferentially with safranin (which has an affinity for highly sulfated mucopolysaccharides like heparin) and the fluorescent dye berberine sulfate (which also binds to heparin), and were ultrastructurally homogeneous in size and electron density, while mast cells identified in the glandular stomach mucosa stained preferentially with alcian blue and were unstained

by berberine sulfate. Mast cell phenotype, therefore, may be functionally regulated at the level of the tissue microenvironment in which a multipotent mast cell precursor or intermediate develops.

Epilogue

Despite an apparent wealth of literature available on the subject, the potential still exists for scholarly, significant contributions to the body of knowledge which describes mast cells. The lineage relationship between the mast cells found in vivo (mucosal and serosal) is still poorly defined, ond only recently have preliminary studies approached the relationship between the aforementioned cells and their putative correlate, the culture-derived mast cell. Little is known of the phenotype of the cells which give rise to mast cells in culture, and the ontogeny of the mast cell in early embryonic tissues is documented in scant and unsystematic reports.

In the course of the remaining chapters of this dissertation, we describe our recent contributions to the study of the mast cell. Beginning with the observation of cell lines with basophilic granules, we have characterized Abelson murine leukemia virus-transformed mast cell-like lines of midgestational, embryonic origin using panels of monoclonal antibodies as well as biochemical and molecular biological techniques (Chapter II). Although mast cells were not detected in homologous, uninfected tissues,

culture-derived mast cells could be propagated from embryonic sources in the presence of exogenously supplied interleukin 3. These studies (and parallel experiments on adult bone marrow-derived mast cells) also provide the first detailed analysis of hematopoietic marker expression of cultures progressing from heterogeneous to homogeneous populations of mast cells (Chapter III). We have subsequently analyzed the frequency of mast cell precursors in embryonic placenta, nonplacental embryonic tissues, and adult tissues, demonstrating the earliest reported mast cell precursors as well as a heretofore unreported rich source of such cells, the placenta (Chapter IV). In the same chapter, we have characterized the cell surface of mast cells grown in semisolid agar media and have presented encouraging preliminary results of experiments designed to sort mast cell precursors on the basis of differentiation antigen expression.

CHAPTER II
ABELSON MURINE LEUKEMIA VIRUS-INFECTED CELLS FROM MIDGESTATION PLACENTA EXHIBIT MAST CELL AND LYMPHOID CHARACTERISTICS

Introduction

Abelson murine leukemia virus (A-MuLV) is a replication-defective transforming retrovirus which was isolated from a tumor in a steroid-treated BALB/c mouse inoculated with the Moloney murine leukemia virus (Abelson and Rabstein, 1970). Molecular analysis of the A-MuLV genome has revealed that the virus arose by recombination between the thymotropic Moloney virus genome and a cellular gene termed c-abl (Goff et al., 1980; Shields et al., 1979). The recombinant virus can infect and immortalize hematopoietic cells in vivo and in vitro, and can transform certain fibroblast cell lines in vitro (Scher and Siegler, 1975). The virus has demonstrated the ability to transform in vivo mature cells of the B lineage (Potter et al., 1973; Premkumar et al., 1975) as well as those of the T (Cook, 1982), myelomonocytic (Raschke et al., 1978; Ralph et al., 1976), and mast cell (Risser et al., 1978; Mendoza and Metzger, 1976) lineages. In contrast, in vitro infection of adult or embryonic primary hematopoietic tissues followed by clonal selection in semisolid media results in the immortalization of cells exhibiting characteristics of the earliest stages of B lymphoid differentiation (Rosenberg et al., 1975; Rosenberg and Baltimore, 1976b;

Boss et al., 1979; Siden et al., 1979; Alt et al., 1981). Abelson virus can also induce agar colony-forming cells which express erythroid characteristics (Waneck and Rosenberg, 1981); the latter cells, however, fail to proliferate as permanent cell lines in liquid culture.

Based upon Abelson virus's propensity to immortalize B lineage precursors in vitro, experiments were designed to study early embryonic lymphoid precursors. The midgestation embryonic placenta has been reported to be the earliest source of B cell precursors (Melchers and Abramczuk, 1980; Melchers, 1979) in the mouse. The successsful development of permanent cell lines from midgestation embryonic placenta transformed in vitro by A-MuLV has recently been reported (Siegel et al., 1985). Primary agar colony counts indicated that the frequency of A-MuLV targets is highest at ten days of gestation. Unlike previously reported A-MuLV-transformed embryonic cell lines, the genomes of the placental cells contain a germline immunoglobulin heavy chain locus characteristic of nonlymphoid cells and perhaps very immature lymphoid cell precursors.

We proceeded to analyze this novel group of A-MuLV embryonic transformants to better ascribe them to cells of a particular lineage. This chapter summarizes our efforts to characterize the A-MuLV transformants derived from midgestation embryonic tissues and presents several

significant observations. First, unlike previously reported Abelson virus-transformed embryonic cells, the placental cell lines isolated in our laboratory exhibit many characteristics of culture-derived and mucosal mast cells including differentiation antigens, high affinity receptors for IgE, and metachromatic granules containing histamine and sulfated proteoglycans. Second, we describe the development of a simple, sensitive, nonisotopic, nonfluorometric method to detect membrane receptors for immunoglobulins. Third, all of the cell lines analyzed had at least one, and sometimes more than one integrated A-MuLV provirus. Finally, the cell lines proliferated independent of exogenous mast cell growth factor, and no growth factor could be detected in either cell lysates or the supernatants of exponentially growing cultures maintained at high cell density.

Materials and Methods

Cell Lines

Cloned A-MuLV-transformed embryonic cell lines were established in our laboratory as previously reported (Siegel et al., 1985). All had been adapted to growth in liquid culture and had been successfully maintained in 10P (RPMI 1640 (GIBCO, Grand Island, NY) with 5×10^{-5} M 2-

mercaptoethanol (Sigma Chemical Co., St. Louis, MO)) and 10 percent heat-inactivated fetal bovine serum (Sterile Systems, Logan, UT)) for at least two years with twice weekly passages prior to analysis. The nomenclature used to designate each cell line included the number of days of gestation (detection of vaginal plug on day 0) followed by a suffix ("P" for placenta of (BALB/cAN x B10.BR/SgSn)F1 origin, "PC" for placenta of (BALB/cAN x CBA/J) origin, and a clone number. Cell line 10P12, for example, was the twelfth clone isolated from 10 day placental cells derived from matings of BALB/cAN females and B10.BR males.

Mouse tumor cell lines used as experimental controls in the studies were chosen to represent the major hematopoietic lineages:

1. B lymphoid

a. FLEI-4, an pre-B cell line derived by E.J. Siden by infection of day 15 BALB/c fetal liver with A-MuLV strain P120;

b. 18-81 (Siden et al., 1979), a pre-B cell line induced by infection of bone marrow cells with A-MuLV strain P120;

2. T lymphoid

a. RLσ 11, a radiation-induced leukemia obtained from N. Rosenberg;

b. B2-4-4, a Moloney virus-induced leukemia obtained from N. Rosenberg;

c. YAC-1, a Moloney leukemia virus-induced lymphoma obtained from R. Weiner (University of Florida);

3. Monocyte-macrophage

a. WEHI-3, a myelomonocytic leukemia obtained from M. Norcross (University of Florida);

b. P388D1, a methylcholanthrene-induced monocytic tumor obtained from American Type Culture Collection (Rockville, MD);

4. Basophil-mast cell

a. P815, a methylcholanthrene-induced mastocytoma obtained from S. Noga (University of Florida);

b. CB6ABMC4, an A-MuLV-induced mastocytoma obtained from M. Potter (National Cancer Institute, Bethesda, MD);

c. BALABMC20, an A-MuLV-induced mastocytoma obtained from M. Potter.

All of the above cell lines were maintained in 10P with twice-weekly passage.

Cell line DA-1, which was used in the analysis of interleukin 3 production, was generously supplied by J.N. Ihle (National Cancer Institute, Frederick, MD) and was maintained in a one-to-one mixture of WEHI-3 conditioned media and enriched media (EM; Razin et al., 1984a).

Analysis of Histamine Content

Histamine content was determined by a modification of the enzymatic-isotopic microassay of Taylor et al. (1980). Histamine methyltransferase (HMT) reagent was prepared from BALB/cAN mouse brain which was homogenized in iced 5mM sodium phosphate buffer, pH7.9 (10 ml per gram of brain) with an iced, sintered glass homogenizer. The crude homogenate was transferred to an Oak Ridge type tube and cleared centrifugally at 50,000xg for ten minutes at 4°C. Histamine standard solutions and tumor cell lysates were prepared in PIPES-BSA which contained 25mM PIPES, pH 7.4 (Sigma Chemical Co., St. Louis, MO), 0.4 mM magnesium chloride, 5 mM potassium chloride, 120 mM sodium chloride, 1 mM calcium chloride, 5.6 nm glucose (Fisher Scientific Co., Fairlawn, NJ), 0.1 percent BSA (Sigma); standard solutions and cells were boiled for ten minutes and cleared for five minutes in a microfuge (Brinkmann Instruments, Westbury, NY). Immediately before starting the reaction, HMT-SAMe was formulated: 500 microliters of HMT reagent was mixed with 10 microliters (5 microcuries) S-[methyl-^{3}H]-adenosylmethionine (76.1 Ci per mMol; New England Nuclear, Boston, MA) and 5 microliters unlabeled S-adenosylmethionine (SAMe, Sigma Chemical Co.; 16 micrograms per ml). Each reaction mixture consisted of 10 microliters of HMT-SAMe and 20 microliters of histamine standard solution or tumor cell lysate. Following incubation for

two hours at 37°C, each reaction was stopped with 0.1 ml of 1 M sodium hydroxide and saturated with solid sodium chloride. The contents of each tube were extracted with 0.25 ml chloroform in the presence of 10 microliters of unlabeled SAMe (16 micrograms per ml)and the phases were separated by brief centrifugation. The aqueous phase was carefully removed with a pasteur pipet and the organic phase was re-extracted with an equal volume of 1 M sodium hydroxide and unlabeled SAMe. Aliquots (150 microliters) of the organic phase were added to toluene-based scintillation fluid (10 ml) and counted on a Beckman liquid scintillation counter (Beckman Instruments, Palo Alto, CA). All samples and standards were prepared in duplicate or triplicate. Histamine content was determined by comparison of tritiated counts in dilutions of tumor lysates to those in histamine standard solutions and were expressed as nanograms (ng) per 10^4 cells.

Cytological Staining

Staining procedures were performed on air dried cytocentrifuge (Shandon Scientific Company, Ltd., London, U.K.) preparations on 25x75 mm precleaned glass slides. Slides were fixed in methanol for one to two minutes prior to staining with either Wright's Giemsa (Schalm et al., 1975) or May-Gruenwald Giemsa (Thompson and Hunt, 1966). Metachromatic cell granules were identified by staining for five minutes with 0.1% w/v toluidine blue in 30% v/v

ethanol, pH 0.5 after fixation for two minutes in Mota's fixative (lead subacetate in acidic ethanol)(Yam et al., 1971). All stained slides were coverslipped with Permount (Fisher Scientific Co.) prior to observation by light microscopy.

Antibodies

Lineage-specific determinants on the surfaces of placental and control cell lines were probed with a panel of monoclonal (mAb) and polyvalent antibodies whose significant features are summarized in Table II-1. All monoclonal antibody preparations, unless otherwise noted, were used as filtered hybridoma supernatants from stationary phase cultures grown (in our facility) in Dulbecco's Modified Eagle's Minimal Essential Media (GIBCO) with 10 percent heat-inactivated fetal bovine serum and $5x10^{-5}$ M 2-mercaptoethanol. Preliminary work with mAb B23.1 was performed with partially purified antibodies generously supplied by P. LeBlanc (University of Florida); later studies were performed with filtered hybridoma supernatants which had been grown in RPMI 1640 with 10 percent fetal bovine serum, generously supplied by G. Place in the laboratory of S. Russell (University of Florida). Monoclonal antibody FT-1 was generously supplied by M. Kasai (National Institute of Health, Tokyo, Japan); the nature of its production and processing have been previously reported (Kasai et al., 1983).

Table II-1. Lineage-Specific Antibodies Used in Surface Marker Analysis

Designation (isotype)	Specificity (Literature Citation)
14.8 (IgG2b)	200 Kd Ly5 antigen on lymphoid cells from spleen, lymph node, and bone marrow (Kincade et al., 1981b).
RA3-2C2 (IgM)	Ly5 antigen on lymphoid cells from spleen, bone marrow, lymph node, and plasma cells but not on thymocytes or CFU-S (Coffman and Weissman, 1981a).
RA3-3A1 (IgM)	220 Kd Ly5 variant (B220) on B lineage cells on spleen, lymph node, and bone marrow, but not on thymocytes (Coffman and Weissman, 1981b).
M6 (IgM)	Probably recognizes Dolichus biflorus agglutinin receptor on early fetal thymocytes and on some thymic leukemia cells (Kasai et al., 1983).
anti-Asialo GM1 (polyclonal)	Neutral glycolipid asialo GM1 on early fetal thymocytes, some fetal liver cells, and few adult bone marrow, spleen, and lymph node cells as well as some thymic leukemia cells and natural killer cells. Not on adult and embryonic, Thy-1 positive thymocytes (Habu et al., 1980; Kasai et al., 1980; Young et al., 1980).
T24/31.7 (IgG)	Thy 1 glycoprotein on thymocytes and T cells from spleen but not on bone marrow prothymocytes (Dennert et al., 1980).
5H1 (IgM)	Abelson transforming antigen on bone marrow targets of A-MuLV, on most thymocytes, some bone marrow and spleen cells, fetal liver erythrocytes and pre-B cells, and bone marrow pre-B cells, but not on lymph node cells, CFU-S or stem cells committed to myeloid lineages (Shinefeld et al., 1980).
B23.1 (IgM)	Antigen on resident and elicited macrophages, adherent cultured bone marrow cells and macrophage-like cell lines as well as on culture-derived mast cells but not on resident peritoneal mast cells (Katz et al., 1983; Leblanc et al., 1982).

Rabbit anti-asialo GM1 was received from W.W. Young (University of Virginia Medical Center) as a delipidated serum. Rabbit anti-rat immunoglobulins was purchased as a lyophilized powder (IgG fraction of rabbit anti-rat IgG (heavy and light chains), Miles Laboratories, Elkhardt, IN) and reconstituted per manufacturer's specifications. Rabbit anti-mouse immunoglobulins was purchased from Gateway Biologicals (St. Louis, MO).

Normal rat serum was prepared from cardiac blood of an unimmunized animal. Normal mouse serum was prepared from pooled specimens of multiple unimmunized BALB/cAN mice.

Cell Surface Markers

Cell surface differentiation antigens were detected by a modification of the method of Uchanska-Ziegler and colleagues (1982). Formalin-treated, heat killed Staphylococcus aureus (S.aureus) Cowan I (The Enzyme Center, Inc.,Boston, MA, and later a generous gift of Dr. Michael D.P. Boyle) were first coated with rabbit anti-rat IgG (RAMIgG, Miles Laboratories, Elkhart, IN) and then with rat monoclonal antibodies specific for mouse differentiation markers. Either hybridoma cell culture media or partially purified immunoglobulins were used as a source of the latter antibodies.

Mouse cells ($1x10^5$ or fewer) selected for analysis were pelleted in round-bottom, PVC microtiter wells (Dynatech, Alexandria, VA) and resuspended in a small

volume (5 microliters) of S.aureus-RAMIgG-monoclonal antibody sandwiches. Following a thirty-minute incubation on ice, the contents of each well were washed six times and a sample was prepared as a cytocentrifuge mount. Slides were stained with May-Gruenwald Giemsa or Wright's Giemsa.

Fc Receptor Assays

Receptors for the Fc domain of mouse IgE and IgG were detected by a novel modification of the S. aureus method used to detect other surface antigens (Siden and Siegel, 1986). Indicator bacteria were prepared by incubating 25 microliters of packed S. aureus or Escherichia coli (E. coli) with 0.175 ml of 2,4,6-trinitrobenzene sulfonic acid (3.7 mg per ml in 0.28M cacodylate buffer, pH6.9) for 10 minutes at room temperature. The 1.5 ml microfuge tube containing the reactants was wrapped in foil to retard photodecomposition and taped to a rotator during incubation. After four washes with 0.01 M phosphate-buffered 0.15 M sodium chloride, pH 7.3 (PBS; Mishell and Shiigi, 1980), the TNP-S. aureus or TNP-E. coli were resuspended to their orginal volume in balanced salt solution (BSS; Mishell and Shiigi, 1980) with 10 mM HEPES (pH 7.35), 0.1% w/v sodium azide, $5x10^{-5}$ M 2-mercaptoethanol (Sigma Chemical Co.) and 1% v/v fetal bovine serum (H10BNF1).

Hybridoma supernatants containing mouse IgE anti-$(DNP)_2$ (IgELa2, American Type Culture Collection) and

partially purified mouse IgG anti-TNP (generous gift of M. Rittenberg, Oregon Health Center University) were cleared by centrifugation at 12,000xg for 15 minutes at 4°C. The latter was diluted in Dulbecco's modified Eagle's minimal essential medium (GIBCO) with 10% v/v heat-inactivated fetal bovine serum, and both contained 0.1% w/v sodium azide. Cells to be analyzed were suspended in H10BNF1 at $1x10^6$ per ml, aliquoted at 0.1 ml per well of a 96-well PVC cluster (Dynatech, Inc., Alexandria, VA) and pelleted by centrifugation (2 minutes, 100xg, 4°C). The cluster was "flicked" and briefly vortexed (five to ten momentary touches) and the cells were resuspended in 0.1 ml of cleared IgE or IgG anti-TNP. Clusters were covered with plastic wrap and placed in a 37°C, 5% carbon dioxide incubator for one hour. Following incubation, the treated cells were washed twice with H10BNF1 by centrifugation. The cell pellets were dispersed by vortexing and 5 microliters of 10% w/v TNP-S. aureus or TNP-E. coli were added to each well. The covered microtiter clusters were placed on ice for thirty minutes and the contents of the wells were washed six times as previously described. The pellets were resuspended in 0.1 ml of H10BNF1. Ten to thirty microliter samples of each well were cytocentrifuged, stained with May-Gruenwald Giemsa and observed by bright field microscopy.

Mice

BALB/cAn mice used in these studies were bred in our colony. B10.BR/SgSn and CBA/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). For studies involving timed pregnancies, females were placed in the cages housing one or two males at ratios of one to three females per male. Females were observed for vaginal plugs following overnight cohabitation and the date of detection was noted as day zero of gestation.

Analysis of A-MuLv Proviral Integration

High molecular weight DNA was extracted from tumor cells by the method of Steffan et al. (1979). Tumor cells were harvested from liquid culture by centrifugation and washed in cold PBS. The cells were resuspended at ten million per milliliter in TES (10 mM Tris, 5 mM EDTA, 100 mM sodium chloride, pH 7.5) and added dropwise to an equal volume of lysis buffer consisting of TES with one percent w/v SDS (Sigma Chemical Co.) and 0.04 percent Proteinase K (Fisher Scientific Co.). The lysates were digested overnight at 37°C with gentle mixing. DNA was extracted from the lysate, twice for thirty minutes with an equal volume of glass-distilled phenol and twice again with chloroform containing 4 percent isoamyl alcohol (Fisher Scientific Co.). The extracted DNA was precipitated in 2.5 volumes of 95 percent ethanol or isopropanol, dissolved in T10E1 buffer (10 mM Tris, 1 mM EDTA, pH 7.5), and dialyzed

extensively against the same buffer. Normal embryonic tissue, prepared in an identical manner, provided control DNA. All DNA was quantitated spectrophotometrically by the OD_{260}/OD_{280} method (Maniatis et al., 1982).

Ten micrograms of DNA were incubated with 10 units of restriction endonuclease BAM HI (New England Biolabs, Beverly, MA) in TA buffer (O'Farrell et al., 1980); completeness of digestion was monitored by the addition of one microgram of bacteriophage lambda DNA to a duplicate sample. The digested DNA was mixed with sample buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 25% w/v Ficoll, 0.05% w/v bromophenol blue, at 5X concentration) and electrophoresed for eighteen hours at 40 volts D.C. on an 0.8% agarose gel in TEA buffer (40 mM Tris-acetate, 2 mM EDTA, pH 7.8). Both the gel and the electrophoresis buffer contained 0.5 micrograms of ethidium bromide per milliliter. Samples were organized such that a set of digested cellular DNAs were pipetted into wells on one side of the gel and duplicates containing bacteriophage lambda DNA were pipetted into wells on the other side of the same gel.

Following electrophoresis, the gel was photographed under ultraviolet light to visualize the restricted DNA and verify that all of the lambda DNA had been digested to completion. A ruler placed alongside the gel was photographed at the same time to provide a scale of DNA fragment sizes for later reference. The lanes containing

lambda DNA were generally cut away and discarded. The gel was then exposed to shortwave ultraviolet light for an additional ten minutes to break the DNA and thereby facilitate transfer. DNA in the gel was denatured with 0.5 M sodium hydroxide, 0.6 M sodium chloride for one hour, neutralized in 1 M Tris, pH 7.4, 1.5 M sodium chloride (two changes of 150 to 200 milliliters for 30 to 45 minutes each), and transferred to nitrocellulose (Schleicher and Scheull, Keene, NH) by the method of Southern (1975).

Probes for A-MuLV-related sequences were prepared from the virus-specific recombinant plasmid pAB3Sub3 (Goff et al., 1980) by nick translation (Rigby et al., 1977) to a specific activity of 10^8 dpm per microgram. The ^{32}P-labeled sequences were hybridized to the nitrocellulose-immobilized DNA for twenty hours at 68°C by the method of Wahl (1979). The nitrocellulose blot was then washed extensively under stringent conditions (0.015 M sodium chloride, 0.0015 M sodium citrate at 68°C). The probed blots were autoradiographed on XAR-5 film (Eastman-Kodak, Rochester, NY) with two calcium-tungstate-phosphor intensifying screens (Cronex Lightning Plus, E.I.DuPont de Nemours and Co., Wilmington, DE) for two to five days.

Conditioned Media

Embryonic tumor cells were cultured under conditions similar to those used to generate WEHI-3 conditioned media (W3CM) (Razin et al., 1984a). Cells from log phase

cultures were seeded at $1x10^6$ per ml in 10P in bacterial-grade petri dishes (Fisher Scientific Co.). Following four days (92 to 100 hours) of incubation in humidified five percent carbon dioxide, the conditioned media were harvested by centrifugation and filtered through tissue culture grade 0.2 micron nitrocellulose filters. Conditioned media were concentrated by stirred-cell ultrafiltration (Amicon, Danvers, MA), by dialysis against polyethylene glycol 20,000 (Fisher Scientific), or by ammonium sulfate (Fisher Scientific Co.) precipitation when such procedures were desired.

Cell lysates were prepared from cells grown to 7 to $9x10^5$ per ml as follows: Cell cultures were centrifuged (ten minutes at 200xg, 4^0) and the cells were washed twice in PBS. The cells were resuspended in one milliliter 10P and subjected to three rounds of alternate freezing (in dry ice-ethanol) and thawing (at 37^oC). The lysates, which contained no viable cells upon microscopic examination, were cleared centrifugally at 2000xg for fifteen minutes (4^oC) and at 12,000xg for fifteen minutes (4^oC), and were filtered through 0.45 micron sterile, disposable filters (Gelman Sciences, Ann Arbor, MI). Lysates were stored at -20^oC prior to use.

Assay for Interleukin 3

Interleukin 3-like activity was analyzed by a modification of the method of Razin and colleagues (1984a).

The proliferation of cell line DA-1 (generously provided by J.N. Ihle), which requires interleukin 3 for growth, served as an assay for interleukin 3. DA-1 cells were centrifuged, washed twice in PBS, and resuspended at $5x10^5$ per ml in appropriate condititioned media or cell lysates. One-tenth milliliter aliquots were pipeted in triplicate into separate compartments of a 96-well microtiter cluster (Linbro, Flow Laboratories, McLean, VA). The cells were incubated (37°C, five percent carbon dioxide) for sixteen hours, at which time each well was pulsed with one microcurie of ^{3}H-TdR (5 Ci per mMol, Amersham Corporation, Arlington Heights, IL) in ten microliters of EM. Following six hours additional incubation, the cells were collected on Whatman glass microfiber filter strips (Whatman Paper Ltd., Maidstone, U.K.) in water by multiple automated sample harvester (MASH, Otto Hiller Company, Madison, WI). The filter strips were air dried. ^{3}H-TdR which was incorporated into filter-immobilized DNA was counted in toluene-based scintillation fluid in a liquid scintillation spectrometer.

Results

Histamine Content of Transformed Placental Cells

Initial light microscopiic examination of some of the transformed cell lines revealed large mononuclear cells

with basophilic granules which stained metachromatically with acidic toluidine blue (H.R. Katz, personal communication). Since the cells fit the working definition of mast cells by these criteria, we began our characterizations by assaying for intracellular histamine. All but two of the placental cell lines analyzed contained histamine as detected by a sensitive, isotopic-enzymatic microassay (Table II-2). The quantity of histamine detected in the cells (5 to greater than 500 nanograms per million cells) was similar to that found in cultured mast cells derived from mouse spleen (450 to 500 nanograms per million cells; Razin et al., 1981a), bone marrow (80 to 150 nanograms per million cells; Razin et al., 1982a), and fetal liver (200 to 1400 nanograms per million cells; Nabel et al., 1981), and mucosal mast cells (160 to 2000 nanograms per million cells; Befus et al., 1982b; Bienenstock et al., 1982), but one order of magnitude less than that found in serosal mast cells (15 micrograms per million cells; Bienenstock et al., 1982). Histamine was not detected in lymphoid or myelomonocytic cell controls, nor was it detected in the mastocytoma P815, which reportedly has variants which are devoid of mast cell granules (Mori et al., 1979). Histamine biosynthesis was confirmed by chromatographic identification (Galli et al., 1976) of [^{3}H]-histamine in extracts of [^{3}H]-histidine-labeled cells (data not shown).

Table II-2. Histamine Content of Embryonic Tumor Cell Lines and Control Tumor Cell Lines

CELL LINES	HISTAMINE ($ng/10^4$ cells)[a]
PLACENTAL CELL LINES	
9P1	>5.00
10P2	2.70
10P6	<0.05
10P8	0.05
10P12	0.06
11PO-1	<0.05
11P62	1.60
CONTROL CELL LINES	
WEHI-3	<0.05
RL♂11	<0.05
18-81	<0.05
FLEI-4	<0.05
P815	<0.05

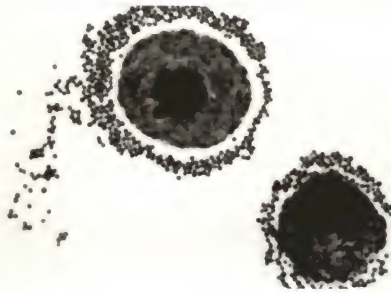
a: Histamine content was determined as described in Materials and Methods.

Analysis of Hematopoietic Lineage Markers

In view of the morphological and biochemical similarity of the placental cell lines to mucosal and culture-derived mast cells, we sought to further confirm the relationship by analysis of the cell surface differentiation antigens with an antibody which discriminated between serosal and culture-derived mast cells (Katz et al., 1983). This and other cell surface antigens were analyzed by a simple and sensitive S. aureus-antibody sandwich method (Uchanska-Ziegler et al., 1982). Figure II-1A shows the binding reaction of the cell line 10P12 with bacteria coated with anti-cultured mast cell antibody B23.1, while Figure II-1B shows that the same cell line, incubated with bacteria coated with unimmmunized rat immunoglobulin, resulted in no bacteria bound to the mouse cell surfaces.

Cell surfaces of the remaining A-MuLV placental transformants and of a number of control tumor cell lines were probed with monoclonal antibody B23.1 as well as a panel of other monoclonal and conventional antibodies reported to be specific for hematopoietic differentiation markers (Table II-1). Table II-3 summarizes the results of the cell surface analyses. Every virus-transformed embryonic cell line expressed the B23.1 epitope, which is

A



B

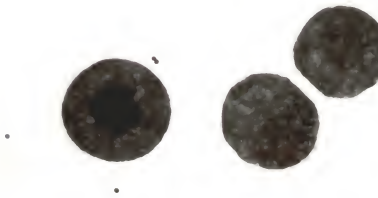


Figure II-1. Detection of Cell Surface Determinants on Abelson Murine Leukemia Virus-Transformed Embryonic Cells.

Killed Staphylococcus aureus bacteria coated with anti-mast cell/monocyte B23.1 antibodies (A) and normal rat serum antibodies (B) were reacted with cells from line 10P12 as indicated in Materials and Methods.

Table II-3. Analysis of Lineage-Specific Surface Determinants on A-MuLV-Transformed Embryonic and Control Tumor Cell Lines*

	PRE-B MARKERS			PRE-T MARKERS		T MARKER	A-MuLV TARGET	MAST CELL -MONOCYTE MARKERS
CELL LINE	RA3-3A1 IgM	14.8 IgG2b	RA3-2C2 IgM	M6 IgM	Anti-Asialo GM1 Polyclonal	T24/31.7 IgG	5H1 IgM	B23.1 IgM
					PLACENTAL CELL LINES			
9P1 Ab-MuLV PLACENTA (9D)	-	-	-	-	+	-	-	+
10P2 Ab-MuLV PLACENTA (10D)	-	-	-	-	-	-	-	+
10P6 Ab-MuLV PLACENTA (10D)	-	-	-	-	++	-	-	+
10P8 Ab-MuLV PLACENTA (10D)	-	-	-	-	-	-	-	++
10P12 Ab-MuLV PLACENTA (10D)	+	+	++	-	-	-	-	++
11PO-1 Ab-MuLV PLACENTA (11D)	++	++	++	+	++	-	-	+
11P62 Ab-MuLV PLACENTA (11D)	-	-	-	-	-	-	-	++

Table II-3. Continued

	CONTROL CELL LINES							
WEHI-3 MYELOMONOCYTIC LEUKEMIA	-	-	-	-	-	++	-	+
RLσ 11 THYMOMA	-	+	++	-	-	++	++	-
B2-4-4 THYMOMA	-	+	+	ND	ND	++	+	-
YAC-1 THYMOMA	ND	ND	ND	++	++	ND	ND	ND
18-81 Pre-B	+	++	++	ND	+	-	++	-
FLKI-4 Pre-B	++	++	++	-	+	-	++	-
P815 MASTOCYTOMA	++	+	++	-	-	-	-	++
CB6ABMC4 MASTOCYTOMA	-	-	+	++	-	-	ND	+
BALABMC20 MASTOCYTOMA	-	-	+	++	+	-	ND	+

* Cell surface determinants were analyzed as described in Materials and Methods.

Scoring: - indicates less than 5 bacteria per mouse cell
+ indicates 5 to 50 bacteria per mouse cell
++ indicates more than 50 bacteria per mouse cell
ND indicates reactivity not analyzed

also found on cells of the monocyte lineage (Leblanc et al., 1982) as well as on culture-derived mast cells. This marker was also detected on control mastocytoma (P815) and myelomonocytic leukemia cells (WEHI-3).

Two of the cell lines expressed three related antigenic determinants of the 200 to 220 kilodalton surface glycoprotein family which is found on mouse lymphoid cells. The cell lines 11P0-1 and 10P12, as well as all of the control lymphoid and adult-derived mastocytomas which we examined, expressed the antigens defined by the monoclonal antibodies 14.8 and RA3-2C2. These epitopes are expressed on B cells and their surface immunoglobulin-negative precursors. The progenitor cells react with the monoclonal antibody RA3-3A1 as well. The latter antibody, however, did not recognize a 220 kilodalton glycoprotein on tumor cells of the T lineage which was detected by 14.8 and RA3-2C2. The selective reactivity of RA3-3A1 has been confirmed previously (Coffman and Weissman, 1981b). It is interesting to note, however, the novel expression of this previously characterized B cell differentiation antigen on mastocytoma P815 as well as our embryo-derived mast cell lines.

Placental and control tumor cell lines were also analyzed for the expression of other primitive lymphoid markers. The neutral glycolipid asialo-GM1, which is expressed on the early embryonic, Thy-1-negative thymocytes

(Habu et al., 1980) and for the expression of receptors for the Dolichos biflorus agglutinin, which is expressed on early fetal thymocytes and on some thymic leukemia cells (Muramatsu et al., 1980). The monoclonal antibody M6 (Kasai et al., 1983) reportedly recognizes cells bearing receptors for Dolichos biflorus agglutinin and probably binds to the receptor itself. Table II-3 shows that only one of the embryonic cell lines, 11P0-1, expressed the early thymocyte differentiation marker recognized by anti-FT-1. The same cell line also bears surface asialo-GM1, as do two other embryonic cell lines, 9P1 and 10P6. It is also interesting to note that two A-MuLV-induced adult mouse mastocytomas, CB6ABMC4 and BALABMC20 were both positive for FT-1, and that the latter cell line also expressed the neutral glycolipid asialo-GM1. These observations will be discussed later in this chapter.

Despite the presence of markers specific for early T lineage cells on several embryonic cell lines, mature T lineage markers were only detected on three control cell lines. Lack of reactivity with monoclonal antibody T24/31.7 (Dennert et al., 1980) indicated that none of the cell lines expressed the Thy-1 differentiation marker. Also conspicuously absent from A-MuLV-transformed embryonic cell lines was the antigen recognized by the monoclonal antibody 5H1, which is expressed on Abelson murine leukemia virus transformation-sensitive targets from mouse bone

marrow as well as on thymocytes, fetal liver red blood cells, and pre-B cells (Shinefeld et al., 1980; E. Siden and L. Shinefeld, unpublished observations).

Analysis of Receptors for IgE and IgG

High affinity membrane receptors for the Fc regions of immunoglobulins were detected with a sensitive, isotype-specific assay developed in this laboratory (Siden and Siegel, 1986). Coated bacteria were prepared by derivatizing the bacterial surfaces with the hapten trinitrophenol (TNP) and then reacting the modified bacteria with mouse monoclonal anti-TNP antibodies of the IgE or IgG classes. Experimental and control cell lines were incubated with the immunoglobulin-coated bacteria at 37^{o}C for one hour and processed for cytocentrifugation and staining. Typical positive and negative reactions are illustrated in Figure II-2. Observations are summarized in Table II-4. IgE receptors were detected on all but two of the embryonic cell lines analyzed, while none of the cell lines expressed high affinity receptors for IgG. Receptors for IgG were detected, however, on the myelomonocytic leukemia cell line WEHI-3 (Table II-4) and on the macrophage-like tumor cell line P388D1 (data not shown). The results of the bacterial assay for IgG receptors were duplicated by the method of Schrader (1981), which used xenogeneic (rabbit) 7S antibodies immobilized on sheep red

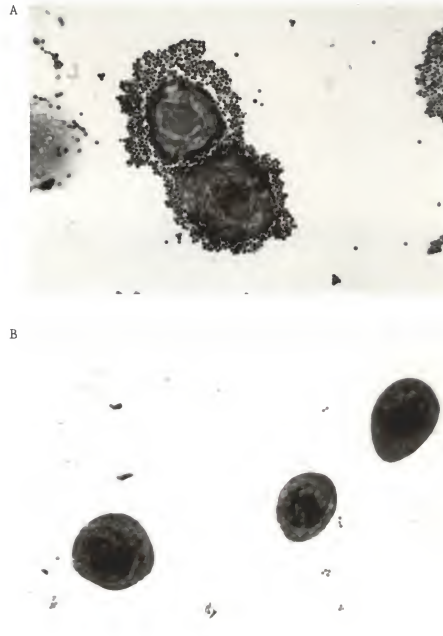


Figure II-2. Detection of Surface Receptors for IgE on A-MuLV-Transformed Embryonic Cells.

Killed Staphylococcus aureus bacteria haptenated with trinitrophenol (TNP) were incubated with cells from line 10P8 which had previously been incubated with monoclonal mouse IgE (A) and IgG (B) as described in Materials and Methods.

Table II-4. Analysis of Surface Membrane Receptors for IgE and IgG on A-MuLV-Transformed Embryonic Cell Lines and on Control Tumor Cell Lines

CELL LINE	MEMBRANE RECEPTORS FOR		
	Mouse IgE[a]	Mouse IgG[a]	Rabbit IgG[b]
PLACENTAL			
9P1	+	-	-
10P2	+	-	-
10P6	-	-	-
10P8	+	-	-
10P12	+	-	-
11P0-1	-	-	-
11P62-4	+	-	-
CONTROL			
WEHI-3	-	+	+
RL 11	-	-	ND[c]
18-81	-	-	-
FLEI-4	-	-	ND
P815	-	-	ND
CB6ABMC4	-	-	ND
BALABMC20	+	-	ND

a: Membrane receptors for allogeneic (mouse) IgE and IgG were detected by the TNP-bacteria/anti-TNP method (see Materials and Methods).
b: Membrane receptors for xenogeneic (rabbit) IgG were detected by the rabbit anti-sheep RBC/SRBC method of Schrader (1981).
c: ND indicates analysis not performed.

blood cells. Rosettes of sheep red blood cells on IgG Fc receptor-positive cells were detected by phase microscopy after staining with crystal violet.

Metachromatic Granules in Transformed Cell Lines

Initial examination of several of the embryonic cell lines, which provided the first evidence for their relationship to mast cells, was followed by a more detailed study of the remaining cells. Cells were fixed in Mota's lead subacetate and stained in acidic toluidine blue to detect granules rich in basophilic glycosaminoglycan. As seen in Table II-5, the majority of the original (BALB/c x B10.BR)F1 embryonic cell lines did not possess metachromatic granules, although two cell lines with high histamine content (9P1 and 11P62) did express the characteristic. Additionally, four of six embryonic cell lines derived from a different paternal background, and all long-term culture-derived mast cells (see Chapter III), stained metachromatically with toluidine blue. Many of the A-MuLV-transformed embryonic cell lines synthesize and secrete chondroitin-4,6-disulfate proteoglycan (D. Levitt, R. Porter, and E. Siden, manuscript in preparation), in support of our observations.

Analysis of A-MuLV Provirus Integration

Although the embryonic tumor cell lines were derived from cells infected with Abelson murine leukemia virus, we sought to confirm the presence of A-MuLV-specific sequences

Table II-5. Metachromatic Granules in A-MuLV-Transformed Embryonic Cell Lines and Control Tumor Cell Lines.

Cell Line	Metachromatic Granules[a]
placental	
9P1	+
10P2	-
10P6	-
10P8	-
10P12	-
11P0-1	-
11P62	+
10PC1	+
11PC14	+
11PC19	-
11PC20	-
11PC32	+
12PC1	+
control	
WEHI-3	-
18-81	-
P815	-
CB6ABMC4	-
BALABMC20	+

[a]: Cytocentrifuged smears of cells were stained with toluidine blue as indicated in Materials and Methods. Cells with metachromatic granules (+) and without metachromatic granules (-) were scored.

in cellular DNA by identifying the proviral genome. DNA isolated from four of the embryonic cell lines was probed with the Abelson virus-specific recombinant plasmid pAB3Sub3. The cellular DNA was first digested with endonuclease BamHI, which recognizes no restriction sites within the virus-specific sequences of the plasmid. Thus, each integrated A-MuLV provirus detected was represented by a single band on the autoradiographed blot. All but one of the cell lines so analyzed showed two copies of the A-MuLV provirus integrated into high molecular weight DNA (Figure II-3); cell line 11P62 showed only one copy. In addition to integrated viral sequences, the probe also detected two germ line fragments of the endogenous c-abl gene, which contains cellular sequences and BamHI sites not present in the plasmid. The v-abl and c-abl patterns of the control cell line 160N54, from which the infecting virus was isolated, are also shown.

Two of the embryonic cell lines, 10P12 and 11P0-1, were injected into mice to determine whether the cells were tumorigenic. Both cell lines caused tumors in syngeneic mice inoculated within two weeks of birth. DNA was prepared from tumors isolated from the mice (output DNA). Restriction analysis of the input (original cell line) and output DNA was performed to determine whether the lesions were due to cell line proliferation or to subsequent infection of host cells by shed virus. Tumor cells derived

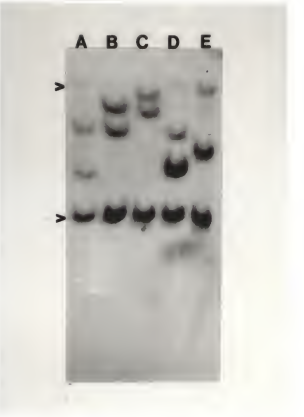


Figure II-3. Virus-Transformed Cells Contain Abelson Murine Leukemia Virus-Specific DNA Sequences.

DNA isolated from embryonic cell lines was restricted, electrophoresed, blotted, and probed with v-abl recombinant plasmid pAB3Sub3, and the filter was autoradiographed as described in Materials and Methods. Lanes contain DNA from control cell line 160N54 (A), 10P8 (B), 10P12 (C), 11P0-1 (D), 11P62 (E). Arrows mark c-abl-containing fragments.

from the input cells would have the same sites of provirus integration, while in vivo virus infection from cells shedding virus would probably result in the observation of different sites, due to the random nature of A-MuLV integration. Both scenarios were observed (Figure II-4). Cells recovered from lymph nodes of mice which were injected with 11P0-1, a virus-producing cell line (E. Siden, personal communication), had viral sequences integrated into cellular restriction fragments distinct from the input DNA pattern. The cell line 10P12, however, which sheds no detectable virus (E. Siden, personal communication), was reisolated from the liver and had A-MuLV-specific sequences integrated into cellular restriction fragments similar in size to the input pattern.

Analysis of Interleukin 3 Production by Embryonic Cell Lines

The phenotypic similarity of the A-MuLV-transformed embryonic cell lines and culture-derived mast cells was sharply contrasted by the growth factor requirements of the respective populations. The former cells required no growth factors beyond those provided by medium 10P (containing fetal bovine serum), while cultured mast cells required an exogenous source of interleukin 3, conventionally provided in WEHI-3-conditioned media. We therefore sought to determine whether the cell lines produced their own growth factor. Using a sensitive

Figure II-4. Tumors Isolated from Mice Injected with Cell Lines 10P12 and 11P0 Contain A-MuLV-Specific DNA Sequences.

DNA was isolated from embryonic cell lines and tumor tissue, restricted with endonuclease Bam HI, blotted onto nitrocellulose, and probed with nick-translated pAB3Sub3 as described in Materials and Methods. Autoradiograph of probed blots is shown. Lanes contain DNA from input cell line 11P0 (A), cultured lymph node tumor cells from animals which were injected with 11P0 (B,C), input cell line 10P12 (D), cultured liver tumor cells from animals which were injected with 10P12 (E,F). Arrows mark c-abl-containing fragments.

proliferation assay, we were unable to detect interleukin 3 in the supernatants or cell lysates of several of the embyonic cell lines, even when the samples were concentrated (Table II-6). Appropriate WEHI-3 conditioned media and cell lysate controls, however, revealed the assay was functional.

Discussion

Despite over one hundred years of systematic study, little is known of the processes of differentiation and maturation of the pharmacologically-active secretory cell termed the mast cell (Ehrlich, 1877). In the broadest sense, mast cells have been associated with a population of mononuclear cells containing biogenic amines and sulfated proteoglycans which are stored in cytoplasmic basophilic granules (Selye, 1965; Metcalfe et al., 1981). The histochemical signature of these cells is the anomalous, metachromatic staining of the granules in the presence of analine dyes (Ehrlich, 1879). Mast cells also have high affinity membrane receptors for IgE, through which, by specific immune reactions, the cells elaborate their biogenic effectors (Austen, 1984).

During the course of the last twenty-five years, a number of reports have established the existence of two

Table II-6. Interleukin 3 Content of Conditioned Media and Cell Lysates of A-MuLV-Transformed Embryonic Cells and Control Tumor Cells[a].

Sample	Stimulation Index[c]		Conditioned Media[d]
IL3 Standard 1[b]	1.000+/-0.000		50% W3CM
			1x W3CM (50% SAS ppt)
IL3 Standard 2	0.836	0.048	1x W3CM (80% SAS ppt)
			1.8x W3CM (Amicon ret)
IL3 Standard 3	0.569	0.064	0.9x W3CM (Amicon ret)
			1x W3CM (Amicon filtrate)
IL3 Standard 4	0.319	0.063	
			100% 10P12-2 CM
IL3 Standard 5	0.146	0.020	10% 10P12-2 CM
			5.5x 10P12-2 CM (80% SAS ppt)
IL3 Standard 6	0.069	0.006	1.4x 10P12-2 CM (80% SAS ppt)
			8.3x 10P12-2 CM (Amicon ret)
IL3 Standard 7	0.042	0.005	1x 10P12-2 CM (Amicon ret)
			1x 10P12-2 CM (Amicon flt)
IL3 Standard 8	0.017	0.004	
			100% 11PC19 CM
IL3 Standard 9	0.010	0.003	50% 11PC19 CM
Blank	0.009	0.002	

Table II-6. Extended

Stimulation Index	Cell Lysates[e]	Stimulation Index
1.384	WEHI-3 (5.2×10^5 C.E.)	0.496
0.061	(2.6×10^5 C.E.)	0.321
0.588	(1.3×10^5 C.E.)	0.174
1.252	(0.6×10^5 C.E.)	0.082
1.242		
0.007		
0.007	10P12-2 (7.3×10^5 C.E.)	0.005
0.007	(3.6×10^5 C.E.)	0.005
0.013	(1.8×10^5 C.E.)	0.004
0.008	(0.9×10^5 C.E.)	0.004
0.006		
0.011		
0.009		
0.007	10P12-2 (7.3×10^5 C.E.):	
0.009	Standard 1 (1:1)	0.764

a. Interleukin 3 content was assayed by a proliferation assay as described in Materials and Methods.

b. Homogeneous interleukin 3 standard was generously supplied by Dr. J.N. Ihle as a concentrate in RPMI 1640 and used to his specifications. Standard 1 was formulated by fifty-fold dilution of the concentrate. Standards 2 through 9 were made by serial two-fold dilutions from standard 1. All dilutions were made in EM, which also served as the blank.

c. Stimulation Index was calculated from raw counts retained on filters by the formula: Stimulation Index= Mean Sample cpm/ Mean Standard 1 cpm, where mean Standard 1 cpm was 46236 (standard deviation, 16129). Stimulation indices for standards (expressed as means +/- 1 standard deviation) were compiled from the results of five experiments of three replicates each, except for Standard 7 which was compiled from three experiments of three replicates. All other stimulation indices were compiled from a minimum of three determinations.

d. Conditioned media were prepared as indicated in Materials and Methods. Abbreviations: CM (conditioned media), SAS (saturated ammonium sulfate), Amicon ret (retentate of 10 Kd cutoff Amicon stirred cell filter), Amicon flt (filtrate of 10 Kd cutoff Amicon filter).

e. Cell lysates were prepared as indicated in Materials and Methods from the number of cells noted parenthetically (C.E. is cell equivalents).

subsets of mast cells which have been contrasted on the basis of morphology, size, thymus-dependent proliferation, fixation and staining requirements, proteases, proteoglycan composition, and histamine content and release (reviewed by Jarrett and Haig, 1984; Shanahan et al., 1984; Katz et al., 1985a). The original mast cells studied by Ehrlich and his proteges have been termed connective tissue or serosal mast cells, while those isolated initially from intestinal mucosa were termed atypical or mucosal mast cells for their aberrant fixation and staining properties (Enerback, 1966a, 1966b). The relationship of the two subsets is still unclear.

A third subset of mast cells was observed with the development of techniques to propagate hematopoietic cell in vitro. The culture-derived mast cells are phenotypically similar to mucosal mast cells by a number of criteria including morphology, granule number, size, and staining requirements, histamine content, and proteoglycan composition. The development of in vitro mast cell precursor culture (Schrader, 1981; Nakahata et al., 1982b; Crapper and Schrader, 1983) has permitted the cultivation of mast cells from a number of adult tissues as well as from day thirteen fetal mouse liver.

Some of the cell lines resulting from the transformation of embryonic placental cells by Abelson murine leukemia virus are morphologically and

histochemically similar to culture-derived mast cells. Analysis of histamine content of the embryonic cell lines indicated that most do contain that biogenic amine. Furthermore, the quantity of histamine detected (5 to more than 500 nanograms per million cells) were similar to those reported for mucosal and in vitro-derived mast cells. The embryonic cell lines also synthesized chondroitin-4,6-disulfate proteoglycan, but not heparin (D. Levitt, R. Porter, and E. Siden, manuscript in preparation). Chondroitin sulfate is a granule constituent of cultured and mucosal mast cells, but not serosal mast cells, which store heparin (Razin et al., 1984a). Based on these two additional criteria, we have proposed that our embryonic cell lines are analogous to culture-derived mast cells.

Our analysis of the surface determinants on embryonic cell lines and control tumor cell lines has supported the preliminary hypothesis of their lineage. Although we observed heterogeneity of histamine content and expression of high affinity receptors for IgE, every embryonic cell line expressed the differentiation antigen recognized by the monoclonal antibody B23.1, which is also expressed on cultured mast cells from mouse bone marrow, spleen, and blood (Katz et al., 1983). Two of the embryonic cell lines described in this study (Table II-3), as well as two embryonic cell lines of different genotype (unpublished results), also express the Ly5 200 to 220 kiltodalton

suface glycoprotein (Omary et al., 1980), which has been observed on cultured mast cells (Nabel et al., 1981) as well as on mastocytomas (Scheid and Triglia, 1979). We have also extended the scope of expression of Ly5 on mast cell tumors to the methycholanthrene-induced P815 and to two A-MuLV/pristane in vivo-induced mastocytomas. Interestingly, the latter three mastocytomas and our Ly5-positive cell lines express the epitope recognized by the monoclonal antibody RA3-3A1, which was previously thought to be B lineage specific (Coffman and Weissman, 1981a, 1981b). Mice homozygous for the mutant lpr gene, which experience severe early onset autoimmune disease, exhibit lymphoproliferation of a thymus-dependent Ly 5-positive, cell population; the proliferating cells, however, lack other B-lymphoid characteristics and appear to be of the T lineage (Morse et al., 1982).

Three of the embryonic cell lines also expressed early thymocyte antigens. One of the cell lines, 11P0-1, expressed the fetal thymocyte-specific epitope FT-1 (Kasai et al., 1983) as well as the neutral glycolipid asialo-GM1, while two other cell lines (9P1 and 10P6) expressed asialo-GM1 only. The latter differentiation antigen, which is expressed on twelve to fifteen day fetal thymocytes (Habu et al., 1980), has also been observed on natural killer cells (Kasai et al., 1980; Young et al., 1980). However, none of the embryonic cell lines exhibited natural killer

activity (M. Jadus, personal communication). The expression of B lineage and T lineage markers, as seen on 11P0-1, may be characteristic of one stage of mast cell differentiation. Alternatively, the expression of B, T, monocyte, and cultured mast cell markers on the Abelson virus-transformed embryonic cell lines may be analogous to that of a yet undefined in vivo multipotent proliferative stem cell which is present in the midgestation placenta. The present studies have not further pursued this matter.

We have characterized cell lines, generated by transformation of embryonic cells by Abelson murine leukemia virus, which phenotypically resemble culture-derived mast cells. However, analysis of nontransformed cells from freshly disaggregated embryonic tissues indicated that few, if any, mast cells are present in the midgestational conceptus (Chapter III). Infection may therefore have transformed the cells prior to stem cell commitment to the mast cell lineage or before committed mast cell precursors can be identified. Undifferentiated, multipotent hematopoietic stem cells, which are responsible for colonization of other fetal tissues, have been described in the mouse embryonic yolk sac blood islands at this stage of development (Moore and Metcalf, 1970).

Abelson murine leukemia virus infection of primary hematopoietic cells followed by culture in semisolid media has previously been shown to generate permanent cell lines

with B lineage characteristics (Rosenberg and Baltimore, 1976; Siden et al., 1979; Alt et al., 1981), and to induce terminally differentiated, erythroid colonies from early placenta and fetal liver (Waneck and Rosenberg, 1981). Although A-MuLV induces mastocytomas in vivo (Mendoza and Metzger, 1976; Risser et al., 1978), and can infect and immortalize cultured mast cells (Pierce et al., 1985; Chapter III of this work), the proliferation of continuous, exogenous growth factor-independent mast cells from midgestation embryonic tissue in the absence of interleukin 3 is unprecedented. Pierce and colleagues (1985) recently reported the generation of interleukin 3-independent mast-like cell lines from day eighteen fetal liver cells infected with A-MuLV and subsequently selected in media containing the growth factor. Those authors speculated that the omission of beta-mercaptoethanol from their tissue culture media was probably responsible for the proliferation of transformed mast cells. Since our cell lines were produced in mercaptoethanol-containing media, we suggest rather that the generation of interleukin 3-independent mast cell lines may be due to unique conditions of transformation which incoporate the B cell mitogen dextran sulfate or the specific targets used.

Abelson murine leukemia virus has been shown to transform, in vitro, macrophages (Greenberger et al., 1979), erythroid cells (Waneck and Rosenberg, 1981), and

pre-B cells (Boss et al., 1979; Siden et al., 1979; Alt et al., 1981) as well as interleukin 3-dependent mast cells (Pierce et al., 1985; Chapter III of this work) and early myeloid lineage cells (Rapp et al., 1985; Cook et al., 1985). All of these cells are sensitive to the proliferative effects of interleukin 3 (Iscove and Roitsch, 1985; Palacios et al., 1984). Our observations, however, are that A-MuLV-infected mast cells do not synthesize detectable interleukin 3 (Table II-6) nor do they contain interleukin 3-specific messenger RNA (E. Siden, personal communication); the former observation has recently been corroborated by others studying Abelson virus-infected mast cells (Pierce et al., 1985) and myeloid cells from more mature stages of development (Cook et al., 1985).

We are presently uncertain of the apparent nonautocrine mechanism of interleukin 3 independence in Abelson virus-infected cells, although several possibilities, including interaction of the v-abl gene product with the interleukin 3 receptor (modifying the activity of the receptor), substitution by the viral gene product for an interleukin 3 receptor intracellular function, or short-circuiting of an interleukin 3-induced proliferation pathway (Pierce et al., 1985) are potentially satisfying. Farrar and colleagues (1985) recently reported that interleukin 3 stimulates the transient redistribution of protein kinase C from the cytosol to the plasma membrane

of interleukin 3-dependent FDC-P1 cells. The protein kinase C translocation kinetics in FDC-P1 cells is paralleled by the DNA synthesis dose response curve of these cells, suggesting a relationship between enzyme association with plasma membranes and cell proliferation. The Abelson murine leukemia virus transforming gene product is a transmembrane protein (Witte et al., 1979b) with known tyrosine protein kinase activity (Witte et al., 1980) and a normal cellular analogue (Witte et al., 1979a). Cells transformed by the virus contain a number of cellular proteins which are phoshorylated on tyrosine residues (Cooper and Hunter, 1981); Sefton et al., 1981a, 1981b). We therefore propose that the Abelson murine leukemia virus transforming protein may act on the substrate of protein kinase C or one of the other enzymes in the pathways that are normally activated by extracellular messengers (like interleukin 3) which generate transmembrane control of cellular functions (Nishizuka, 1984; Marx, 1984; Michell, 1984). Further studies to determine which, if any, of these mechanisms is in effect are in order to elucidate the functional role of the A-MuLV transforming protein.

CHAPTER III
CHARACTERIZATION OF MAST CELLS DERIVED FROM MIDGESTATION EMBRYONIC TISSUES IN LIQUID CULTURE

Introduction

Mast cells have been the object of innumerable scientific investigations since their initial description by Ehrlich (1877). The study of mast cells experienced renewed impetus in the nineteen-sixties based upon research in two major areas. First, the development of in vitro culture techniques confirmed the existence of mast cell precursors in a variety of tissues and indicated that mast cell differentiation was dependent on factor(s) elaborated by stimulated lymphocytes. Secondly, the development of improved cytological fixation and staining techniques (Enerback, 1966a, 1966b) permitted the characterization of a new class of "atypical" mast cells (mucosal mast cells) which were were distinct from the connective tissue-associated (serosal) mast cells studied in detail prior to that time. Within a short period of time, a morphological, biochemical, and functional relationship between the in vitro-derived mast cells and mucosal mast cells was noted.

The distinction between the culture-derived and serosal mast cells was facilitated by the development of a series of monoclonal antibodies which discriminated between the two subsets (Katz et al., 1983). The observation of metachromatic granules in A-MuLV-transformed placental cell

lines suggested that we probe the cells with B23.1 (Chapter II), an antibody which recognizes culture-derived mast cells. These studies led to the observation of other mast cell markers on the transformed cell lines, namely histamine and membrane receptors for IgE.

The expression of mast cell characteristics by A-MuLV transformants derived from murine placenta opened several related areas for investigation. We first desired to learn if precursors to culture-derived mast cells exist in midgestation embryonic tissue. Second, having found embryonic precursors to these mast cells, we wanted to know if the markers expressed on the A-MuLV transformants were also expressed on "normal" cells cultured in interleukin 3. We therefore conducted a detailed analysis of the progression of cell populations in mast cell cultures over the course of several weeks of selection and enrichment. Third, we studied the effects of Abelson virus infection and adherent cell cytokines on long-term cultured mast cells. The scope and significance of our findings are discussed in this chapter.

Materials and Methods

Procedures for the husbandry of mice, detection of cell surface determinants and Fc receptors, and cytological staining were performed as described in Chapter II.

WEHI-3 Conditioned Medium (W3CM)

W3CM was prepared following the method of Razin and colleagues (1984a). WEHI-3 myelomonocytic leukemia cells from log phase cultures were seeded at $1x10^6$ per ml into an enriched medium (EM) consisting of RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% v/v native fetal bovine serum (Sterile Systems, Logan, UT), 2mM L-glutamine, 0.1 mM nonessential amino acids, 100 Units per ml penicillin, 100 micrograms per ml streptomycin (GIBCO), and 0.05 mM 2-mercaptoethanol (Fisher Scientific Co., Medford, MA). Cultures were maintained in one of two ways: 200 ml cultures in 17x150 mm dishes (Falcon Plastics, Oxnard, CA) were incubated at 37°C in a humid, five percent carbon dioxide incubator (Forma Scientific, Marietta, OH); alternatively, 1000 ml cultures were seeded into pregassed EM in polycarbonate roller bottles (Corning Glass Works, Corning, NY) which were placed a horizontal roller apparatus (New Brunswick Scientific, New Brunswick, NJ) in a controlled environment room (37°C). Following four days (92-100 hours) of incubation, the conditioned media were harvested by centrifugation (15 minutes at 2000xg, 4°C) and filtered through tissue culture grade 0.2 micron nitrocellulose membranes (Nalgene, Rochester, NY). Media harvested from small cultures were pooled into 1000 ml lots prior to filtration. W3CM was aliquoted in smaller volumes (100, 250, 500 ml) in sterile bottles and stored for up to

six months at -20°C; volumes for immediate use were stored for up to one month at 4°C. Fresh glutamine was added to all media over one month of age. Unless otherwise noted, W3CM was diluted with an equal volume of EM to yield 50% W3CM.

Preparation of Cell Suspensions for In Vitro Culture of Mast Cells

Bone marrow

Two to three month-old BALB/cAn mice were killed by cervical dislocation. The skinned hind legs were disarticulated at the pelvis and tarsals and were placed in EM. The limbs were transferred to a fresh dish of EM in which most of the flesh was removed from the femorae and tibiae by cutting and teasing with sterile dissection tools. Following transfer of the bones to a third dish of EM, the bones were disarticulated and the ends sheared off. Bone marrow was then harvested by flushing the contents of all four bones with EM (1 ml injected through each end with a 25 gauge needle) through 110 micron mesh nylon screens (Tetko, Inc., Elmsford, NY) into a 50 ml conical polypropylene centrifuge tube (Corning Glass Works) (Siegel et al., 1985).

Spleen

Two to three month-old BALB/cAn mice were killed by cervical dislocation, after which the spleens were removed by careful, asceptic dissection and placed in a dish of EM.

The spleens were transferred to fresh medium and residual fat and membranes were removed. The trimmed spleens were minced with sterile scissors onto nylon screens and dissociated through the screens by massaging the tissue with the rubber end of a plunger of a disposable 3cc syringe. Disaggregated cells were washed through the screen into the underlying centrifuge tube with ten to twenty milliliters of EM.

Embryonic tissues

Embryonic tissues were isolated at the indicated days of gestation after vaginal plugs were observed on day 0. Pregnant mice were killed by cervical dislocation and the gravid uteri were surgically removed and placed in a petri dish containing EM. After two to five minutes (to allow the tissues to bleed into the media) each uterus was transferred to a second dish of EM and the residual mesentery and fat were trimmed off. The uteri were transferred to third dish of EM and concepti were dissected away from the maternal tissues and placed in a fresh dish of EM. Concepti of ten or more days of age were teased into placental and nonplacental embryonic tissue (NPET) components. The ectoplacental cones were dissected free of attached membranes and placed in a fresh dish of EM. Concepti of eight and nine days were processed without further dissection. All tissues were dissociated through nylon screens as previously described.

Establishment of Liquid Cultures of Factor-Dependent Mast Cells

Mast cells were cultured from fresh tissues following modification of a previously published protocol Razin et al., 1984a). Except for variations in the methods used to dissociate the cells from their native structures, as noted above, the procedure for propagating mast cells from each source was the same.

Disaggregated cell suspensions were allowed to settle by gravity at room temperature in conical centrifuge tubes for five to ten minutes. Cells remaining in suspension were transferred to a clean tube and were washed three times in EM by centrifugation. Viable, nucleated cells were enumerated by trypan blue exclusion and the cells were resuspended at a concentration of $1x10^5$ per ml in 50% W3CM. Cultures were maintained in a humidified atmosphere of five percent carbon dioxide in air at 37°C. Mast cells were enriched in liquid culture and selected at weekly intervals by gently swirling the dishes and transferring the suspended cells with a pipet to a centrifuge tube; following centrifugation, the cells were resuspended in 50% W3CM at 1 to $2x10^5$ per ml.

Infection of In Vitro-Derived, Factor-Dependent Mast Cells with Abelson Murine Leukemia Virus

Cultures of mast cells derived from adult and embryonic tissues were infected at various times during the

enrichment-selection period and thereafter. Transforming virus stocks, prepared as previously described (Siegel et al., 1985) by superinfection of Abelson P160 nonproducer cell line 160N54, contained 1 to $2x10^6$ PFU of Moloney murine leukemia virus (M-MuLV) and 0.5 to $1.0x10^6$ of A-MuLV per ml and were stored at -70°C prior to rapid thawing immediately before use. Mast cells were pelleted at 200xg at room temperature for ten minutes and resuspended in A-MuLV virus stock with 4 micrograms Polybrene (Aldrich Chemical Co., Milwaukee, WI) per ml at 0.5 to $4x10^5$ cells per ml. Cells were incubated with virus for two and one-half hours at 37°C (Rosenberg and Baltimore, 1976a) in capped 12x75 mm polypropylene culture tubes (Fisher Scientific Co.) with gentle, end-over-end rotation. Following the adsorption period, the suspension was diluted to a final cell concentration of $1x10^5$ per ml with EM or W3CM (final concentration of 50% W3CM, plus virus stock and EM) and cultured in a humidified atmosphere of five percent carbon dioxide in air. Cells in culture were counted at two to five day intervals and fed weekly by centrifuging the cells and resuspending them at 1 to $2x10^5$ per ml in 50% W3CM or EM, as appropriate. Disaggregated cells from fetal livers dissected from day 18 embryos were infected with A-MuLV and cultured as above.

Immunoprecipitation of Viral Proteins

Viral proteins in A-MuLV-infected cells were identified by immunoprecipitation as previously described (Siden et al., 1979). Virus-infected culture-derived mast cells and control cells were pelleted at 200xg for ten minutes at room temperature and washed once with balanced salts solution (BSS). The cells (1.5 to $2x10^6$) were resuspended at $2x10^6$ per ml in labeling media consisting of RPMI 1640 media without methionine (Flow Laboratories, Rockville, MD), 2 mM glutamine, 1x RPMI vitamins (GIBCO), 100 units penicillin and 100 micrograms streptomycin per ml (GIBCO), 10 mM HEPES (Sigma Chemical Company), pH 7.35, 0.05 mM 2-mercaptoethanol (Sigma Chemical Company), and 78 microcuries of ^{35}S-methionine (1126 Ci per mMol, New England Nuclear, Boston, MA). Cells were incubated in labeling media for two hours at 37°C with gentle rocking. The labeled cells were centrifuged for five minutes at 400xg, washed once with BSS and lysed in 1 ml of phosphate lysis buffer (PLB, 10 mM sodium phosphate (Fisher Scientific Co.), pH 7.5, 100 mM sodium chloride (Fisher Scientific Co.), 1 percent v/v Triton X-100 (Fisher Scientific Co.), 0.5 percent w/v sodium deoxycholate (Fisher Scientific Co.), and 0.1 percent w/v sodium dodecyl sulfate (SDS, BDH Chemicals, Ltd., Poole, U.K.)) which was supplemented with 0.098 percent w/v bovine serum albumin (Sigma Chemical Co.), 1 mM EDTA (Fisher Scientific Co.), 1 mM TAME (Sigma

Chemical Co.), 1 mM PMSF (Sigma Chemical Co.), and 0.0002 percent w/v Aprotinin (Boehringer Mannheim GmbH, Indianapolis, IN). The lysates were cleared at 4°C for ten minutes at 2,000xg and sixty minutes at 45,000 revolutions per minute in a Ti50 rotor of a Beckman L5-50 ultracentrifuge (Beckman, Palo Alto, CA).

Lysates were analyzed for incorporated radioisotope by spotting five microliter volumes on Whatman 3MM paper (Fisher Scientific Co.). The filters were washed in five percent w/v trichloroacetic acid, dried, and counted in a Beckman liquid scintillation spectrometer with toluene-based scintillation fluid. Volumes of labeled lysates, normalized to equivalent numbers of counts, were immunoprecipitated with five microliters of either goat anti-M-MuLV or normal goat serum by incubation over night at 4°C on a rotator, followed by incubation for two to three hours with prewashed, heat-killed S. aureus bacteria (50 microliters at 10 percent w/v) in 1.5 ml microfuge tubes. The contents of the tubes were centrifuged at 12,000xg for thirty seconds and the pellets were washed three times with PLB and resuspended in fifty microliters of sample buffer containing 62.5 mM Tris, pH 6.8, one percent w/v SDS, 50 mM dithiothreitol (Sigma Chemical Co.), 5 mM EDTA, and bromophenol blue (Fisher Scientific Co.). Immunoprecipitated proteins were heated for thirty minutes at 68°C and microfuged for three minutes at room temperature

to pellet the bacteria prior to electrophoresis on a 7.0 percent SDS-polyacrylamide gel with a 5.0 percent stacking gel. Running buffer of 0.6 percent w/v Tris, 2.9 percent w/v glycine, 0.1 percent w/v SDS and 0.114 percent w/v sodium thioglycollate (Sigma Chemical Co.) was used. Following electrophoresis at 150 volts (DC), the gel was stained for fifteen minutes with 0.25 percent w/v Coumassie Brilliant Blue R in aqueous 45 percent (v/v) methanol, ten percent (v/v) glacial acetic acid. The gel was destained over night against several changes of ten percent (v/v) acetic acid with two "Adsorptors" destaining sponges, rehydrated with three, ten minute changes of water, and soaked for forty minutes in 1M sodium salicylate (Fisher Scientific Co.) in water. The gel was sandwiched between a clean sheet of 3MM filter paper and plastic wrap prior to drying under vacuum (BIO-RAD gel drier, Oakland, CA). The dried, fluorographed gel was exposed to XAR-5 film (Eastman-Kodak, Rochester, NY) at -70°C for the time indicated.

Coculture of Long Term Mast Cells with Adherent Cell Underlayers and Adherent Cell Conditioned Media

Bone marrow cells were prepared from normal BALB/cAN tissue isolated as previously described (see Preparation of Cell Suspensions for In Vitro Culture of Mast Cells). Washed cells were resuspended at $1x10^6$ per ml in 50% W3CM and 2.5 ml were aliquoted in each well of a six-well tissue

culture cluster (Costar, Broadway, MA). After six days in culture (five percent carbon dioxide in air at 37°C), the supernatants, with nonadherent cells, were removed and 2.5 ml of fresh 50% W3CM were added. Two days later, the bone marrow-derived adherent cell-conditioned media (bmadhCM) were harvested by centrifugation (200xg, fifteen minutes) and filtration through a PBS-washed, 0.2 micron disposable filter (Gelman Sciences, Inc., Ann Arbor, MI). The adherent cell monolayers were washed twice with cold PBS to remove any residual nonadherent cells and conditioned media and then overlayered with 2 ml of 0.3 percent agar (Difco, Detroit, MI) in 50% W3CM.

P388D1 monocytic tumor cells were grown in RPMI 1640 with 10 percent v/v heat-inactivated fetal bovine serum, 2 mM glutamine and 0.05 mM 2-mercaptoethanol (10P) to a density of $2x10^5$ to $1x10^6$ per ml. Cells were pelleted by centrifugation (200xg for ten minutes at room temperature) and resuspended at $1x10^6$ per ml in 10P made 20 micrograms per ml with Escherichia coli serotype B5:055 lipopolysaccharide (LPS, Sigma Chemical Co.) (10P/LPS) after the method of Lachman and colleagues (1979). Within three hours, most of the cells, which were aliquoted at $1x10^6$, $0.5x10^6$ and $0.25x10^6$ per well in a six well cluster (in 1 ml of 10P/LPS), had become adherent. P388/LPS-conditioned media (P388/LPSCM) were harvested after two additional days in culture in a manner identical to that

described for bmadhCM. Cells from the healthiest, most confluent monolayers (seeded at $1x10^6$ per ml) were washed twice with cold PBS and overlayered with 2 ml of 0.3 percent agar containing 10P/LPS or 50% W3CM.

Exponentially growing cultures of WEHI-3 myelomonocytic cells at $4x10^5$ per ml were centrifuged for ten minutes at 200xg and resuspended at $1x10^6$ per ml in fresh 10P/LPS. Individual compartments of a twelve-well tissue culture cluster (Costar) received $1x10^6$, $0.5x10^6$ or $0.1x10^6$ cells; all volumes were normalized to one ml with 10P/LPS. Cells were cultured for two days under these conditions, at which time the conditioned media were harvested and processed as previously described. Only those wells seeded with $0.1x10^6$ cells had healthy adherent layers and were selected for coculture experiments. The latter cells were washed twice with cold PBS prior to overlayering with 1 ml 0.3 percent agar in either 50% W3CM or 10P/LPS.

Mast cells derived from (BALB/cAN x CBA/J)F1 placenta and BALB/cAN bone marrow were selected and enriched to a homogeneous population as previously described. At day 37 of culture, the cells were prepared for the experiment by centrifugation at 200xg for ten minutes at room temperature. The cell pellet was resuspended at a concentration of $2x10^5$ per ml in 100% W3CM and one ml of the suspension was pipeted into each well of the six-well

clusters used in the adherent bone marrow and P388D1 experiments; alternatively, 0.5 ml of suspension was pipeted into each well of the twelve-well cluster used in the WEHI-3 experiments. Mast cells were cocultured with adherent monolayers prepared as previously described and with control underlayers of 0.3 percent agar in appropriate media but without adherent cells. All suspensions were made 50% W3CM by addition of an equal volume of EM.

The effects of adherent cell-derived factors were investigated by making the mast cell suspensions 50% W3CM by addition of an equal volume of bmadhCM, P388/LPSCM or W3/LPSCM. Control cultures of mast cells were similarly made by addition of equal volumes of EM or 10P/LPS as appropriate. Cultures were fed weekly for three weeks by addition of an equal volume of homologous media and analyzed for surface markers as previously described.

Results

Progression of Lineage Markers in Mast Cell Cultures in Liquid Media

Previous studies have dealt with the expression of surface and cytochemical markers in the cells alternatively called cultured mast cells and P cells, but only after the populations had become homogeneous by successive selection

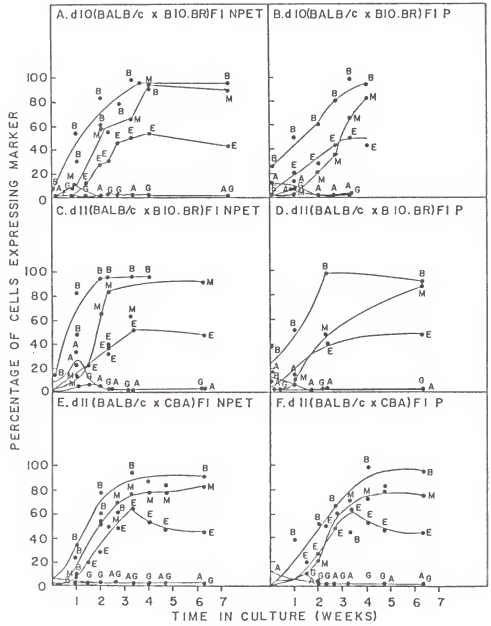
and enrichment, a process requiring at least four weeks in culture. Characteristics such as IgE receptors, metachromatic cytoplasmic granules (Schrader, 1981) and a surface determinant recognized by the monoclonal antibody B23.1 (Katz et al., 1983) have been ascribed to such cells. We chose to analyze the expression of these markers, as well as markers found on A-MuLV-transformed embryonic mast cells, on the heterogeneous populations of cells from which mast cells evolve to better understand the evolution of homogeneous mast cell cultures.

Embryonic tissues

The first significant finding of this study was that mast cells or their precursors exist in the midgestation embryonic placenta and nonplacental embryonic tissues (NPET). The existence of mast cells in such tissues was hypothesized after homologous A-MuLV transformants were characterized (CHAPTER II). As seen in Figure III-1, the number of liquid culture cells with cytoplasmic metachromatic granules (Figure III-2) rose from less than one percent in freshly disaggregated tissues to nearly eighty percent of the cells after four weeks in culture. Concurrently, the number of cells expressing high affinity receptors for IgE rose from less than one percent to approximately fifty percent. A similar time course was followed by the determinant recognized by monoclonal antibody B23.1, which was expressed by nearly all cells in

Figure III-1. Progression of Hematopoietic Lineage Markers in Long-Term Mast Cell Cultures Derived From Embryonic Tissues

Cultures were maintained and analyzed as described in Materials and Methods. Cultures were derived from nonplacental embryonic tissue (NPET, panels A, C, E) and from placenta (P, panels B, D, F) isolated from (BALB/c x B10.BR)F1 (panels A, B, C, D) and (BALB/c x CBA)F1 (panels E,F) concepti at 10(panels A,B) and 11(panels C, D,E,F) days post coitum. Percent cells expressing determinants recognized by monoclonal antibodies RA3-3A1 (A) and B23.1(B), receptors for IgE(E) and IgG(G), and cells with metachromatic granules (M) are indicated.


A. d10(BALB/c x B10.BR)F1 NPET
B. d10(BALB/c x B10.BR)F1 P
C. d11(BALB/c x B10.BR)F1 NPET
D. d11(BALB/c x B10.BR)F1 P
E. d11(BALB/c x CBA)F1 NPET
F. d11(BALB/c x CBA)F1 P
PERCENTAGE OF CELLS EXPRESSING MARKER
0
20
40
60
80
100
1 2 3 4 5 6 7
TIME IN CULTURE (WEEKS)

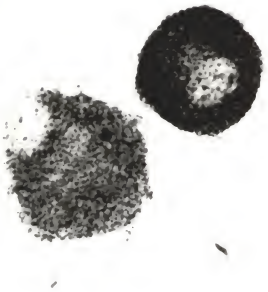


Figure III-2. Metachromatic Granules of Long-Term, Culture-Derived Embryonic Mast Cells.

Mast cells were propagated, cytocentrifuge smears were prepared, and slides were fixed and stained with 0.1 percent acidic toluidine blue as described in Materials and Methods.

culture by the end of the third week. The latter determinant, however, was expressed at significant levels (25 to 25 percent) on freshly dissociated placental cells of (BALB/c x BIO.BR)F1 origin, but not on syngeneic embryo (NPET) cells or on cells derived from (BALB/c x CBA/J) concepti (Figure III-2).

The expression of high affinity receptors for IgG and the lymphoid B-220 determinant recognized by the antibody RA3-3A1 remained relatively insignificant during the course of culture. Expression of markers on mast cells derived from days ten and eleven placenta and NPET were similar; furthermore, there were no qualitative differences between mast cells isolated from (BALB/c x B10.BR)F1 and (BALB/c x CBA)F1 embryonic tissues.

After four weeks in culture, no adherent cells were observed in the dishes and essentially all of the cells contained metachromatic granules as assessed by toluidine blue staining. In addition to expression of the surface determinant recognized by monoclonal antibody B23.1, membrane receptors for IgE (but not IgG), and metachromatic granules, mast cells derived from midgestation murine placenta also expressed the paternal Class I histocompatability antigen (k haplotype), confirming that the precursors to the cells were embryonic, rather than

maternal (data not shown). The expression of Class I markers on P cells was previously reported by Schrader (1981).

Bone marrow

The progression of cell surface and cytochemical markers on bone marrow-derived mast cells was similar to that observed in embryonic-derived tissues (Figure III-3). The number of cells expressing the B23.1 determinant and staining metachromatically with toluidine blue rose dramatically from basal levels in fresh tissue to essentially all of the cells at four weeks, while the number of cells expressing receptors for IgE rose more gradually to approximately fifty percent of the population. At the same time, cells expressing receptors for IgG and the B-220 lymphoid determinant were an insignificant portion of the total cells in culture by two weeks.

Expression of Cell Surface Antigens on Cultured Mast Cells Infected with Abelson Murine Leukemia Virus

Long term liquid cultures of mast cells are characterized by a relatively homogeneous population of cells which express the determinant recognized by the monoclonal antibody B23.1, have receptors for IgE and possess metachromatic granules in their cytoplasm. They are, however, devoid of the B-lymphoid marker recognized by the monoclonal antibody RA3-3A1, which is expressed on some of the A-MuLV transformed placental mast-like cell lines.

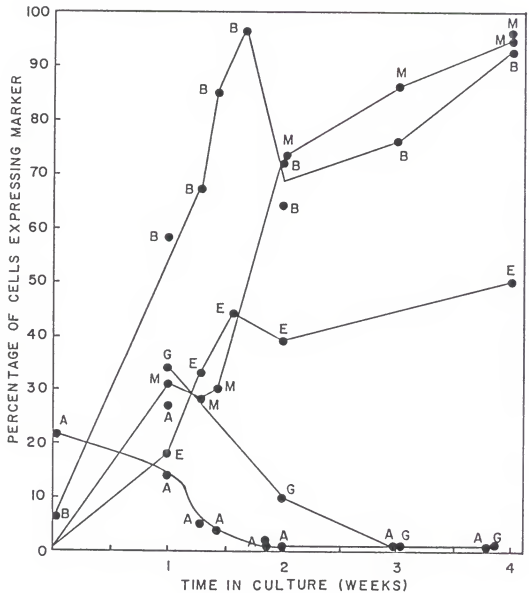


Figure III-3. Progression of Hematopoietic Lineage Markers in Long-Term Mast Cell Cultures Derived From Adult Bone Marrow

Cultures were maintained and analyzed as described in Materials and Methods. Data shown are for BALB/c bone marrow-derived mast cell cultures characterized for expression of determinants recognized by monoclonal antibodies RA3-3A1 (A), B23.1(B), receptors for IgE (E) and IgG (G), and cells with metachromatic granules (M).

We were therefore interested in determining whether A-MuLV infection of culture-derived mast cells could induce the expression of the lymphoid B-220 antigen.

Initial experiments were designed to infect bone marrow-derived mast cells throughout the course of their maturation and to analyze the surface marker distribution of the population. Cells infected one, two, and three weeks after initial isolation and culture were considered "transitional" in that the target population was heterogeneous with respect to the surface and cytological markers assayed; similarly, cells four weeks or later in the culture period were relatively homogeneous (Figure III-3).

Following A-MuLV infection, mast cell cultures experienced a decrease in population size attributed to virus-related killing of some infected cells (Figure III-4). The effects were more dramatic in virus-infected cultures which were grown in the absence of growth factor and were attributed to the inability of factor-dependent cells to proliferate in EM alone, as demonstrated by the short half-life of uninfected mast cells which were grown in parallel cultures without W3CM (Figure III-4). The period of attrition peaked within the first four days of culture post-infection and was followed by an equally dramatic period of proliferation of surviving cells; parallel

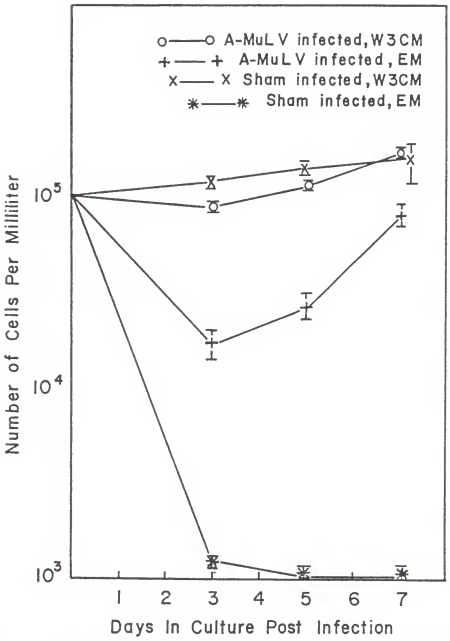


Figure III-4. Population Dynamics of Bone Marrow-Derived Mast Cells Infected with A-MuLV

Long-term (at least 28 days) bone marrow-derived mast cells were infected with A-MuLV or sham-infected and subsequently grown in 2.5 ml cultures with or without W3CM.

cultures infected with M-MuLV or sham infected (incubated with fresh DME with 4 micrograms Polybrene per ml) did not proliferate if deprived of growth factors.

The expression of the lymphoid B-220 antigen was analyzed as a function of primary culture age. Initially, populations of cells which had been in culture for one, two, three, or four weeks prior to infection were probed twenty-one days post infection. The data from these experiments are summarized in Figure III-5. Populations of cells infected at early time points during the development of mast cell cultures displayed similar proportions of cells expressing the B-220 determinant as uninfected populations, while at late times the cells retain the B-220-negative phenotype of the cells in liquid culture (Figure III-3). The cell culture systems, however, were different by one important criterion. In the standard procedure used to propagate culture-derived mast cells, only nonadherent cells are passaged each week, thereby selecting against the adherent population. In the virus- and sham-infected cell cultures, however, the nonadherent cells were maintained in the presence of the adherent cells present in the original culture (and their progeny). Thus, more adherent cells were present in cultures infected at one week than at later times. Adherent cells supported the growth of lymphoid cells as well as culture-derived mast cells. The RA3-3A1-positive population is probably

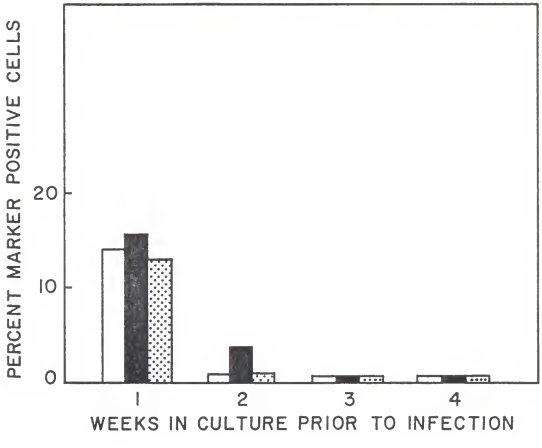


Figure III-5. Expression of Ly5 Antigen on A-MuLV-Infected Mast Cells

Bone marrow cells were cultured under conditions favorable for selection and enrichment of mast cells and infected with A-MuLV at the times noted above. Uninfected cells were assayed at the time of infection (unshaded bars) and parallel cultures of infected (shaded bars) and sham-infected (stippled bars) were assayed twenty-one days post infection for the presence of Ly5 with monoclonal antibody RA3-3A1 as described in Materials and Methods.

transformed bone marrow pre-B cells which are present in the early cultures. This was substantiated by the morphology of the cells in the cultures. Figure III-6 shows both RA3-3A1-positive agranular, lymphoid cells and RA3-3A1-negative granular cells in the same day 28 culture.

Subsequent infections of mast cells with A-MuLV were performed with long-term cultures which were deficient in both adherent cells and RA3-3A1-positive cells. When assayed as described above, neither A-MuLV- nor sham-infected culture-derived mast cells had constituent subpopulations expressing the lymphoid marker (data not shown).

The presence of A-MuLV in cultures of cells treated with the virus was assayed to confirm that the cells were indeed productively infected with the same transforming virus which provided us with the placental tumor cell lines described in Chapter II. Biosynthetically ^{35}S-methionine-labeled cells were lysed in detergent with proteinase inhibitors and the cleared lysates were specifically precipitated with goat anti-M-MuLV. The latter antiserum precipitates all major M-MuLV proteins and will likewise bind to the A-MuLV gag-abl transforming protein by nature of its M-MuLV gag epitopes. The immunoprecipitated proteins were separated electrophoretically on a 7.0 percent, SDS polyacrylamide gel. The autoradiograph of the gel (Figure III-7) shows virus specific bands of

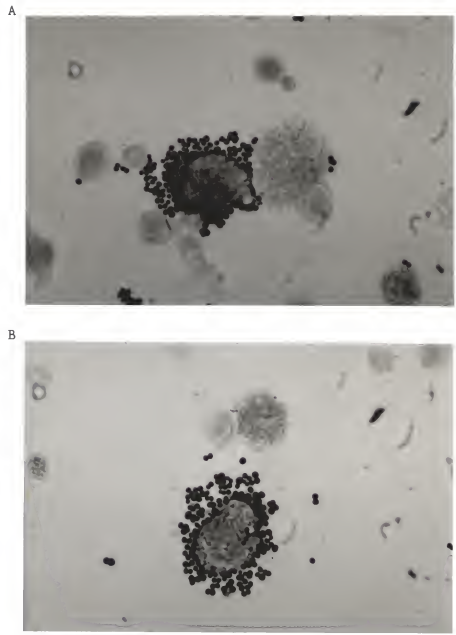


Figure III-6. Mixed population of RA3-3A1-Positive Lymphoid Cells and RA3-3A1-Negative Cultured Mast Cells in Long-Term Bone Marrow Cultures.

Bone marrow cells were cultivated for one week and infected with A-MuLV (A) or sham infected (B) as described in Materials and Methods. After three additional weeks of culture, cells were probed with S. aureus-RA3-3A1 as previously described.

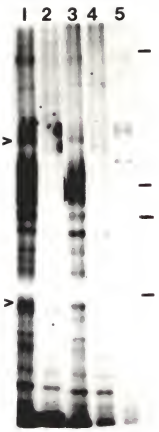


Figure III-7. Abelson Murine Leukemia Virus-Infected Mast Cells Express v-abl Gene Product.

Proteins in A-MuLV-infected cells and controls were biosynthetically labeled and immunoprecipitated with goat anti-Moloney MuLV prior to SDS-polyacrylamide gel electrophoresis, fluorography, and autoradiography as described in Materials and Methods. Electrophoresis standards used were (bars on right, from top to bottom) myosin (205 kilodaltons, Kd), beta-galactosidase (116 Kd), phosphorylase B (97.4 Kd), and bovine serum albumin (66 Kd). Lanes 1, 3, and 5 were loaded with immune precipitated cell lysates; lanes 2 and 4 were loaded with lysates adsorbed with normal goat serum. Lanes 1 and 2, A-MuLV-transformed placental cell line 10P12; lanes 3 and 4, A-MuLV-transformed culture-derived mast cells; lane 5, sham-infected culture-derived mast cells. Upper arrow indicates v-abl gene product; lower arrow indicates major Moloney-MuLV gene product (Pr65).

approximately 160 and 65 kilodaltons (Kd) in lanes loaded with immune precipitated lysates from the placental tumor cell line 10P12 (lane 1), from A-MuLV infected fetal liver (lane 3) and from A-MuLV infected embryonic mast cells (lane 5). The same bands are absent from the lanes loaded with nonimmune (normal goat) serum-treated lysates of the same cells (lanes 2, 4 and 6, respectively) and from a lane loaded with immune precipitated lysate from sham-infected mast cells (lane 7). We conclude, therefore, that the mast cells were productively infected with A-MuLV. Furthermore, the infection of culture-derived mast cells with A-MuLV did not induce the de novo expression of the lymphoid B-220 marker in those cells.

Effects of Adherent Cells and Adherent Cell-Derived Factors on Populations of Cultured Mast Cells

Because conditions which involved coculture with adherent cells generated mast cells expressing Ly 5, we tried to recreate this milieu using bone marrow adherent cells, cell lines, or conditioned media. Expression of B-220 was assayed with RA3-3A1 antibody as previously described. As seen in Table III-1, neither the cocultivation with adherent cells nor their conditioned media with homogeneous populations of mast cells was sufficient to induce the expression of B-220. The expression of the antigen recognized by the B23.1 antibody, receptors for immunoglobulin IgE and the presence of

Table III-1. Effect of Cocultivation of Culture-Derived Mast Cells with Adherent Cells and Their Conditioned Media.

Mast Cell Treatment[a]	Percent Cells Expressing[b]			
	RA3-3A1 Epitope	B23.1 Epitope	IgE Receptors	Metachromatic Granules
day 11 Placenta-derived mast cells[c] cocultured with:				
50% W3CM[d] only	8	95	55	72
LPS-induced P388 cell underlayer				
with 10P/LPS in agar	4	93	52	70
with 50% W3CM in agar	<1	99	51	66
Agar only underlayer				
with 10P/LPS in agar	2	98	54	69
with 50% W3CM in agar	<1	92	49	68
No underlayer				
+50% W3CM + 50% 10P/LPS	3	99	47	69
+50% W3CM + 50% P388CM	4	97	45	67
50% W3CM only	2	96	55	72
LPS-induced WEHI-3 underlayer				
with 10P/LPS in agar	<1	94	ND	ND
with 50%W3CM in agar	3	90	46	73
Agar only underlayer				
with 10P/LPS in agar	2	92	45	80
with 50% W3CM in agar	<1	89	42	71
No underlayer				
+50% W3CM + 50% 10P/LPS	<1	94	47	72
+50% W3CM + 50% W3/LPSCM	2	96	48	74
50% W3CM	2	100	52	67
Adherent bm cell underlayer				
with 50% W3CM	8	97	48	53
No underlayer				
+50% W3CM + 50% bmadh CM	5	98	49	67

Table III-1. Continued

Mast Cell Treatment	Percent Cells Expressing			
	RA-3A1 Epitope	B23.1 Epitope	IgE Receptors	Metachromatic Granules
bone marrow-derived mast cells[e] cocultured with:				
50% W3CM only	<1	99	56	100
Adherent bone marrow cells underlayer with bmadh CM in agar +50% W3CM + 50% bmadh CM	<1	99	50	93
Adherent bone marrow cell underlayer with 50% W3CM in agar	<1	100	52	96
Agar underlayer with bmadh CM	2	93	ND	+
No underlayer + bmadh CM	<1	95	60	98

a. Long term (four weeks and older) mast cell cultures were cocultured with adherent cells in agar underlayers, with cell-free agar underlayers and with no underlayers. In some experiments, media other than 50% W3CM was used in formulating the agar (these are prefixed by "with"). In some experiments, the liquid media in which the mast cells were grown was supplemented with adherent cell conditioned media (these are prefixed by "+"). For details see Materials and Methods.

b. Following coculture under the indicated conditions,mast cells were analyzed as described in Materials and Methods. One hundred to two hundred cells were assessed microscopically for each characteristic and the number of characteristic-positive cells was calculated as a percentage of the entire population. In some instances, insufficient cell numbers precluded assay (ND); in one instance, less than one hundred cells were counted, and no percentage was determined of the marker-positive cells (+).

c. Mast cell cultures were derived from embryonic placenta of (BALB/c x CBA)F1 concepti isolated at 11 days post coitum as described in Materials and Methods.

d. Abbreviations used: W3CM (WEHI-3 conditioned media), LPS (bacterial lipopolysaccharide), 10P/LPS (standard medium 10P supplemented with LPS, 20 micrograms per ml), P388CM (conditioned media of cell line P388), W3/LPSCM (conditioned media of cell line WEHI-3 grown in media supplemented with LPS), bm (bone marrow), bmadh CM (conditioned media of adherent bone marrow cells).

e. Mast cells cultures were derived from BALB/cAN bone marrow as described in Materials and Methods.

metachromatic granules remained unchanged as well in mast cells derived from adult bone marrow or embryonic (day eleven) placenta.

Discussion

The observation of mast-like cells in lines derived by the transformation of midgestation embryonic placenta by Abelson murine leukemia virus (Chapter II) prompted a number of questions which were addressed in this chapter: Are there mast cells in the placental and nonplacental embryonic tissues at days ten and eleven of gestation? Are there mast cell precursors in these tissues? Is A-MuLV responsible for the expression of lymphocyte characteristics on mast cells? The results of our experiments have provided new and significant insight to our understanding of mast cell differentiation.

Analysis of dissociated, fresh bone marrow, placenta and nonembryonic placental embryonic tissues indicated few or no cells with toluidine blue-stained metachromatic granules indicative of mast cells. Mast cells, therefore, comprise at most a minor, and perhaps insignificant, portion of the cells in the tissues examined.

We have also documented, for the first time, the presence of mast cell precursors in both embryonic placenta and nonplacental embryonic tissues. Mast cell precursors

are present in significant numbers in both types of tissues derived ten and eleven days post coitum. Previously, Ginsburg and colleagues (1982) reported that mast cells occassionally arose in control cultures of day ten to day thirteen adherent embryonic cells which were generally used for feeder layers in cultures of mouse thymus-derived mast cells. The authors, however, did not provide detailed description of the phenotype of such cells.

Numerous authors have reported that mast cells derived from a variety of adult tissues can be maintained in culture for prolonged periods of time in the presence of required growth factors. Although no attempts were made to establish permanent mast cell lines like those previously reported (Nabel et al., 1981; Nagao et al., 1981; Razin et al., 1981a; Schrader et al., 1981; Tertian et al., 1981), we were able to maintain bone marrow-derived mast cells in vitro for more than seven weeks without appreciable loss of viability (determined by trypan blue exclusion). In contrast, mast cells derived from placenta seldom survived more than five weeks before catastrophic decline in viable cells. Similar results were reported by Ginsburg and colleagues (1982) for culture of adult lymphoid tissue-derived mast cells on embryonic feeder layers. Those authors, however, ascribed the decline in cell number to loss of growth factor, a variable which we have controlled throughout culture. We propose that the embryo-derived

mast cells, in contrast to adult bone marrow-derived mast cells, prematurely become insensitive to the proliferative effects of interleukin 3. These cells may be impoverished of interleukin 3 receptors, perhaps by a capping mechanism similar to that observed in B cells, in which anti-mouse mu chain antibodies induced the disappearance of cell surface IgM in 14 day mouse fetal liver explant and dissociated adult lymphoid tissue cultures (Raff et al., 1975) and inhibited the mitogenic effect of lipopolysaccharide in the absence of 2-mercaptoethanol (Sidman and Unanue, 1978). Alternatively, the embryonic culture-derived mast cells may be defective in a post-receptor molecular mechanism, the nature of which is unknown. We have not further investigated this interesting phenomenon, which requires more detailed examination.

Within one week of initiation of culture, over fifty percent of bone marrow-derived cells express the B23.1 differentiation marker. The proportion of cells expressing this marker continues to increase until, at four weeks, almost all cells are B23.1-positive. Similar population dynamics were observed when the cells were stained with acidic toluidine blue. The proportion of cells which expressed membrane receptors for IgE increased more gradually from background levels in fresh bone marrow to approximately fifty percent of the cells at week four. The latter data are in contrast to a previous report

(Tertian et al., 1981) that most of the cells in long-term cultures of mast cells have IgE receptors. This discrepancy may be the result of differences in receptor assay technique or may result from other experimental variances such as cell cycling which could temporally affect the expression of membrane receptors. We have observed on one A-MuLV-transformed embryonic cell line that the rosetting assay used by Tertian and colleagues, detects more cells with membrane receptors for IgE than the S. aureus assay (data not shown).

The progression of surface markers on cultured embryonic cells (Figure III-1) follows a pattern similar to those observed for bone marrow-derived mast cells (Figure III-3). Freshly disaggregated placenta contains a population of cells which have receptors for normal rat IgG and are recognized by monoclonal antibody B23.1, but few or no such cells bind IgE or have metachromatic granules. Based on previous reports (Katz et al., 1983), these cells are probably related to the monocyte-mononuclear phagocyte lineage and may be the cells responsible for the binding and degradation of anti-paternal antibodies (Raghupathy et al., 1984). Fewer cells expressing the B23.1 epitope are found in the nonplacental embryonic tissues of (BALB/c x B10.BR)F1 concepti. The development of metachromatic granules and IgE receptors in cultures of cells derived from embryonic tissues was delayed (perhaps because the

precursors were more immature) in contrast to bone marrow-derived mast cells cultures (Figures III-1 and III-3) for the first week of in vitro growth but subsequently reached similar proportions by week four. Expression of the B23.1 epitope, however, demonstrated similar population dynamics for both adult and embryonic tissue-derived cells following the first week in culture.

Reports of the progression of markers and cell types in mast cell cultures have previously lacked quantitative data of the kind presented here. In a series of studies spanning two decades, Ginsburg (Ginsburg, 1963; Ginsburg and Sachs, 1963; Ginsburg and Lagunoff, 1967; Davidson et al., 1983) presented observations of sequential changes in population morphology, size, granule content, nucleus-to-cytoplasm ratio, and mitotic activity of cultures of mouse thymocytes on feeder layers. The four stages of in vitro mast cell development began with small colonies of primitive mast cells and progressed to large lymphocytes (or mastoblasts), young mast cells with mitotic figures, and amitotic mature mast cells. Ishizaka et al. (1976) reported that although less than 0.05 percent of similarly prepared rat thymocytes initially possessed receptors for IgE, most of the cells at the end of one week in culture were blasts which had variable numbers of granules but were still incapable of binding IgE. IgE receptors were not detected in the Ishizaka cultures before day fourteen.

Having established the presence of mast cell precursors in tissues identical to those used for A-MuLV transformation, we infected "differentiated, long-term culture-derived mast cells with A-MuLV to determine whether such cells would be induced for the expression of the RA3-3A1 determinant. The infected cells were phenotypically identical to uninfected cells grown in the presence of interleukin 3 with respect to metachromatic granules and expression of the B23.1 epitope. The B lymphoid Ly5 marker found on two of the original A-MuLV-transformed placental cell lines (Chapter II) was not detected on either infected or uninfected mast cells, although it was detected on nongranular lymphoid cells in heterogeneous early cultures of bone marrow cells which had been infected with A-MuLV (Figures III-5, III-6). The RA3-3A1-positive cells were probably Abelson virus-transformed pre-B cells, the precursors of which are absent in long-term, homogeneous mast cell cultures.

Our observations that Abelson murine leukemia virus can infect mature cultured mast cells and abrogate their requirement for interleukin 3 have recently been corroborated in the literature. Pierce and colleagues (1985) reported that fetal liver-derived mast cells, infected with A-MuLV and maintained in media containing interleukin 3 for three weeks, subsequently formed colonies at high efficiency in the absence of the growth factor.

The A-MuLV-infected, fetal liver-derived mast cells were phenotypically similar to uninfected cells with respect to morphology, presence of metachromatic granules, 20-alpha-hydroxysteroid dehydrogenase, and high affinity receptors for IgE. Attempts to establish factor independent mast cell lines with BALB-murine sarcoma virus (MSV), Harvey-MSV, and Moloney-MSV have been unsuccessful (Pierce et al., 1985). Abelson virus has also been shown to abrogate the interleukin 3-dependence of the early myeloid cell line FDP-1 and to similarly release the interleukin 2-dependent cytotoxic T cell line CTB6 (Rapp et al., 1985; Cook et al., 1985). The mechanism by which the Abelson transforming protein releases our cell lines, and those reported elsewhere, from growth factor requirement is not understood; it would appear, however, to be independent of autocrine effects (Cook et al., 1985; Pierce et al., 1985; Rapp et al., 1985).

Although the expression of the Ly5 differentiation marker on cultured mast cells has been previously reported (Nabel et al., 1981; Tertian et al., 1981; Wong et al., 1982), the detection of the B lymphoid variant of Ly5 on A-MuLV-transformed mast cells (Chapter II) and on mast cells picked from agar colonies (Chapter IV), but not on liquid culture-derived mast cells (Chapter III) in this laboratory was enigmatic. The cells which expressed the RA3-3A1 epitope had, in common, maturation in agar in association

with adherent cell monolayers which grew in the presence or absence of WEHI-3 conditioned media. Early reports of mouse mast cell culture noted the absolute requirement of such monolayers for mast cell differentiation and proliferation (Ginsburg, 1963; Ginsburg and Sachs, 1963; Ginsburg and Lagunoff, 1967). Ishizaka and colleagues (1976) observed that rat thymus-derived mast cells grew more rapidly in the presence of a feeder layer, while Davidson and colleagues (1983) reported that fibroblasts were required for mast cell granule synthesis.

In conclusion, we have demonstrated the presence of mast cell precursors in the murine placenta and nonplacental embryonic tissues during days ten and eleven of gestation. The mast cells generated in liquid culture are phenotypically similar to some Abelson virus-transformed cell lines derived from the same tissue as well as cultured mast cells derived from other tissues. We have described the evolution of homogeneous mast cell cultures from heterogeneous uninfected tissues in terms of histochemical and surface marker expression. Finally, we have demonstrated that while A-MuLV can infect mature mast cells and abrogate their requirement for interleukin 3, the virus does not induce the ectopic expression of the Ly 5 differentiation antigen which is expressed on some of the Abelson virus-transformed mast-like cell lines.

Neither cocultivation with adherent cells nor their conditioned media resulted in the expression of the B220 antigen on long-term cultured mast cells (Table III-1), while the expression of receptors for IgE, the epitope of monoclonal antibody B23.1, and the presence of metachromatic granules was unchanged with respect to untreated mast cells. We therefore conclude that neither the adherent cells used nor their cytokines are responsible for the expression of Ly5 on the surface of mature mast cells. Further studies are required to clarify the Ly5 expression enigma. It seems likely that Ly5 may only be transiently expressed on mast cells during the course of their differentiation. We propose that the expression of the determinant is perhaps fixed when such cells are transformed with Abelson murine leukemia virus and protracted when immature mast cells are maintained in agar culture. Alternatively, the B-220 variant of the Ly 5 antigen may be expressed on a more differentiated form of mast cell which is not present in standard liquid cultures.

CHAPTER IV
ISOLATION, ENUMERATION, AND CHARACTERIZATION OF *IN VITRO* MAST CELL PRECURSORS DERIVED FROM MIDGESTATION EMBRYONIC PLACENTA

Introduction

The understanding of the relationships between the various components of the hematopoietic system has greatly benefited from the development of clonal assay systems during the last quarter century. From the seminal work of Till and McCulloch (1961), the concept of a pluripotent hematopoietic stem cell (CFU-S), capable of clonally reconstituting the spleen and bone marrow of lethally irradiated mice, led to the paradigm that all of the cellular elements of blood were related by a single progenitor. The rapid evolution of *in vitro* clonal culture techniques has further contributed to our understanding of normal and pathogenic hematopoiesis. Techniques are presently available for quantitation of pluripotent hematopoietic precursors from a variety of sources (Johnson and Metcalf, 1977; Hara and Ogawa, 1978; Fauser and Messner, 1979). In addition, methods for isolation, differentiation, and enumeration of committed, multipotent hematopoietic precursors have been described for a number of lineages.

The *in vitro* quantitation of mast cell precursors in a variety of tissues has thus been accomplished by limiting dilution in liquid culture (Crapper and Schrader, 1983;

Guy-Grand et al., 1984) as well as clonal cultures in semisolid media (Schrader et al., 1981; Zucker-Franklin et al., 1981; Nakahata et al., 1982b). All of these methods exploit the ability of mast cell precursors to proliferate and differentiate in the presence of a factor now termed interleukin 3 (Ihle et al., 1981) which has been isolated from media conditioned by lectin- or antigen-stimulated T lymphocytes as well as several permanent cell lines (reviewed by Clark-Lewis et al., 1985; Ihle, 1985; Iscove and Roitsch, 1985; Schrader et al., 1985; Yung and Moore, 1985). Under such conditions, mast cell precursors have been found in a variety of tissues including adult bone marrow, spleen, thymus, lymph nodes, gastric and intestinal mucosa, blood, neonatal cord blood, and fetal liver (reviewed by Katz et al, 1985a; Austen, 1984; Jarrett and Haig, 1984).

In previous chapters we have characterized the mast-like, A-MuLV-transformed murine cell lines derived from midgestation embryonic placenta (Chapter II) and the subsequent detection of precursors of mast cells from similar uninfected tissues (Chapter III). We now report the isolation and quantitation of mast cell precursors from embryonic tissues of mid- and late gestation. A number of significant and novel findings are described. First, we have described mast cell precursors at the earliest reported time in embryonic mouse development. Second, the

midgestation placenta and nonplacental embryonic tissues (NPET) are a rich reservoir of mast cell precursors, containing proportionately at least as many such cells as adult bone marrow. Third, in characterizing the cells in mast cell colonies, we have found that they express a variant of the Ly5 differentiation antigen previously reported to be specific for B lymphocytes. Finally, we describe preliminary experiments which differentiate mast cell precursors from other bone marrow elements on the basis of surface membrane determinants. These findings provide significant new information about mast cell differentiation and ontogeny. Based upon our findings, we discuss the role of mast cells and their precursors in terms of murine embryonic hematopoiesis and the immunobiology of the maternal-fetal interface.

Materials and Methods

Procedures for the husbandry of mice, detection of cell surface determinants and Fc receptors, and cytological staining were performed as described in Chapter II. The procedure for the preparation of WEHI-3 conditioned media (W3CM) was performed as described in Chapter III.

Cultivation of Mast Cell Precursors in Semisolid Media

Mast cell precursors were cultivated from fresh

tissues following a modification of the method used to propagate culture-derived mast cells in liquid suspension culture. Disaggregated cells were prepared from tissues as described in Chapter III. Washed, enumerated cell suspensions from various tissues were resuspended at twice the concentration desired for the most concentrated innoculum in 50% W3CM. The cells were then aliquoted into 12 well tissue culture clusters (Costar, Broadway, MA) in volumes necessary to provide the total number of cells desired per culture, and the total volume of each well was brought to 0.5 ml with 50%W3CM. BACTO agar (DIFCO Laboratories, Detroit, MI) was prepared in sterile water (Travenol Laboratories Inc., Deerfield, IL) at 3.0% w/v, autoclaved for twenty minutes and cooled to 42 to 45 degrees in a water bath. The agar was added to prewarmed (37^{o}C) 50% W3CM at one part agar to four parts medium and mixed thoroughly by pipeting. One-half ml of the 0.6% agar was added to each well of cell suspension and the contents of each well were mixed well by pipeting. The cultures were then allowed to gel at room temperature for ten to twenty minutes before being transferred to a five percent carbon dioxide incubator.

Cultures were fed by diluting freshly prepared, 42 to 45^{o}C autoclaved agar in prewarmed 50% W3CM at one volume of agar per nine volumes of media. One ml of the 0.3% agar was overlayered onto each well and allowed to gel at room

temperature as described above. Cultures were fed once weekly until the colonies were enumerated and analyzed.

Surface Markers on CFU-MC: Sorting of Mast Cell Precursors with Monoclonal Antibodies

Mast cell precursors (CFU-MC) in adult BALB/cAN bone marrow were screened for the presence of determinants recognized by the monoclonal antibodies B23.1 (Katz et al., 1983) and RA3-3A1 (Coffman and Weissman, 1981b) and for expression of receptors for IgE. Initial experiments designed to define the optimum method for selection of cells with the desired phenotype, when performed with tumor cell controls, indicated that neither panning (Wysocki and Sato, 1978; Kay et al., 1977) nor complement-mediated cytolysis were effective. A procedure for selective depletion of rosetted cells (D. Levitt, personal communication), however, proved effective in achieving the desired goal.

B23.1 and RA3-3A1-positive cell selection

Hybridoma tissue culture fluids were obtained from high cell density cultures, cleared by centrifugation at 2000xg for fifteen minutes, filtered through 0.2 micron membranes, and were stored at 4°C after addition of 0.01 to 0.02 percent w/v sodium azide. The hybridoma supernatants were recleared centrifugally, warmed to room temperature and placed in a beaker with a magnetic stir bar. Sodium sulfate (Fisher Scientific Co.) was added slowly (over the

course of one to one and one-half hours) to the stirring liquid to a final concentration of 9g per 50 ml. The slurry was poured into large polyethylene tubes and centrifuged at room temperature in a J-21C centrifuge with a JA-20 rotor (Beckman, Palo Alto, CA) for fifteen minutes at 5000 RPM. The supernatant was discarded and the pellet was dissolved in a small volume of phosphate-buffered saline (PBS) and dialyzed extensively (two changes of 400 to 1000 volumes) against 0.1M sodium bicarbonate (Fisher Scientific Co.) at 4°C. The protein concentration of the dialysate was estimated by absorbance at 280 nm in a SP8-100 ultraviolet spectrophotometer (Pye Unicam, Ltd., Cambridge, UK) prior to further processing. N-hydroxysuccinyl biotin (NHS-biotin, Sigma Chemical Co.) was dissolved in dimethylsulfoxide (Sigma Chemical Co.) at 1 mg per ml. The sodium bicarbonate dialysate and NHS-biotin were mixed vigorously at 200 micrograms of derivatized biotin per milligram of protein, then incubated for four hours at room temperature on a rotator. The modified antibody preparation was dialyzed extensively against PBS at 4°C and was filtered through a 0.2 micron disposable nitrocellulose filter (Gelman Sciences, Inc., Ann Arbor, MI) into a sterile polypropylene tube (Fisher Scientific Co.) prior to storage at 4°C.

Sheep red blood cells (SRBC, from blood freshly drawn at the J. Hillis Miller Health Center Animal Resources

facilities and diluted with an equal volume of sterile Alsever's solution (Mishell and Shiigi, 1980)) were washed four times by centrifugation (ten minutes, 400xg, room temperature) with sterile 0.15 M saline. The pellet of the final wash was resuspended by flicking the tube and an equal volume of filter-sterilized 0.5 mg per ml egg avidin (Sigma Chemical Co.) was added. Two ml of filter-sterilized 0.1 mg per ml chromic chloride (Fisher Scientific Co.) was added dropwise to the red blood cell-avidin mixture with constant, low speed vortexing over two to three minutes at room temperature in a biological containment hood. The reactants were held an additional five to ten minutes at room temperature and then the avidin-modified sheep red blood cells were washed four times by centrifugation with balanced salts solution (BSS). The cells were resuspended to 5 percent v/v in BSS and stored for up to one week at $4^{o}C$.

Bone marrow cells were prepared from two to four month old BALB/cAN female mice as previously described. After counting the washed cells, $1x10^{6}$ to $3x10^{7}$ cells were pelleted by centrifugation and washed once in cold PBS. The pelleted cells were resuspended in cold, biotin-modified antibody. Pilot studies, using tumor cells rich in the surface markers (11P62-4 for B23.1, 18-81 for RA3-3A1), indicated 0.1 ml of antibody preparation per $1x10^{6}$ cells provided optimum labeling; the same volume to cell

number ratio was used in the depletion experiments. Following a thirty minute incubation at 4°C, the cells were washed three times by centrifugation in cold BSS and resuspended in avidin-modified SRBC at a ratio of 100 SRBC per bone marrow cell. The cells were pelleted for one minute at 200xg (4°C) and then incubated on ice for twenty minutes. The cells were then centrifuged for an additional five minutes, the supernatant was removed and the pellet resuspended by flicking. The cells were overlayered on 3 ml of isotonic Ficoll-Hypaque (Lympholyte M, Accurate Chemical and Scientific Corp., Hicksville, NY) and centrifuged for twenty minutes at 800xg (room temperature). The separation medium and its contained cells were carefully transferred to a second tube, the pelleted rosettes and free SRBC were flicked, and both were washed twice in cold PBS prior to lysis with 0.17M ammonium chloride, 0.01M HEPES, pH 7.35 (Hall, 1981). Alternatively, the density-separated populations were depleted of red blood cells by hypotonic shock (Mishell and Shiigi, 1980) after two washes in room temperature BSS. The red blood cell-depleted, sorted bone marrow cells were washed two times with cold PBS or room temperature BSS (as appropriate) and resuspended in EM for counting. Counted cells were resuspended in 50% W3CM for plating in 0.3 percent agar as previously described.

IgE receptor selection

Media from high density cultures of hybridoma IgELa2 (American Type Culture Collection, Rockville, MD) were cleared by centrifugation and preserved with sodium azide as previously described. The processed supernatants were dialyzed for two days against PBS (three changes of 200 volumes) at 4°C to remove the sodium azide, filter sterilized and stored at 4°C in a polypropylene tube.

Sheep red blood cells in Alsever's solution (see above) were washed four times by centrifugation with PBS/one percent w/v glucose. Haptenating reagent was prepared by dissolution of trinitrobenzene sulfonic acid (TNBS, Sigma Chemical Co.) in 0.28M cacodylate buffer, pH 6.9 at 18.75 mg TNBS per ml and sterile filtration (Mishell and Shiigi, 1980). The sterile TNP-SRBC were prepared by dropwise addition of one volume of the flicked cell pellet into seven volumes of haptenating reagent with constant, gentle mixing. The reagents were pipeted back into the tube previously emptied of the SRBC and then incubated on a rotator for thirty minutes at room temperature. Following centrifugation and removal of the supernatant, the TNP-SRBC were washed in PBS/one percent w/v glucose/one percent v/v heat inactivated fetal bovine serum four to six times, until the supernatants were colorless, resupended to 5 percent by volume and stored at 4°C.

Washed and counted bone marrow cells were incubated with PBS-dialyzed, filter-sterilized IgE anti-DNP_2 hybridoma supernatants; again, 0.1 ml of antibody preparation was used per $1x10^6$ cells (empirically determined to be optimum) and $1x10^6$ to $3x10^7$ cells were sorted at one time. The tubes containing the cells were incubated for one hour at 37°C, centrifuged (ten minutes at 200xg, 4°C) and washed twice with cold BSS. The pelleted cells were flicked, resuspended in heavily-modified TNP-SRBC (in 100-fold excess) and centrifuged one minute at 375xg (4°C). Following a twenty-minute incubation on ice, the tubes were centrifuged at 375xg for an additional five minutes, the supernatants removed and the resuspended pellets carefully overlayered on 3 ml of Lympholyte M. Remaining procedures were identical to those performed on B23.1 and RA3-3A1-sorted cells, with the notable exception that only hypotonic shock was found to be effective at lysing TNP-SRBC.

Multiple sorting experiments were performed as described for single sorting, with the following exceptions: Bone marrow cells were incubated with one antibody preparation, washed three times with cold PBS and then resuspended in the second antibody preparation for an additional thirty minute incubation. Following washes as noted before, the bone marrow cells were mixed with either 200 fold excess of avidin-SRBC or 100 fold excess each of

avidin-SRBC and TNP-SRBC, as appropriate. Further processing was identical to that noted above, with hypotonic shock used to deplete the reactants of red blood cells.

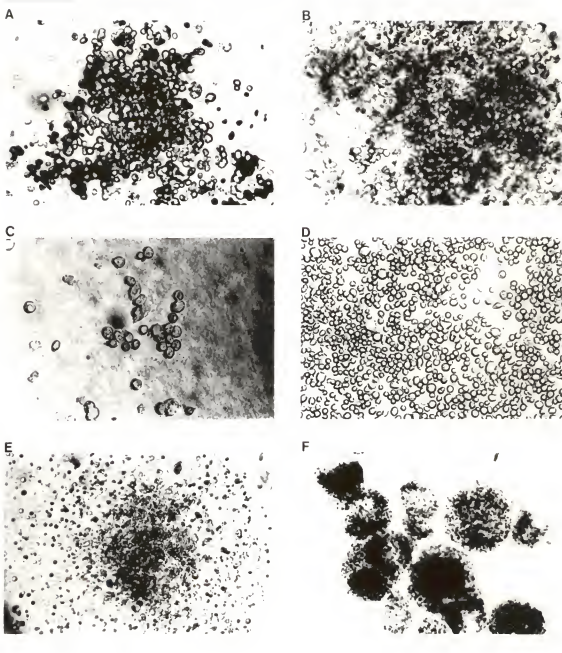
Results

Frequency of Mast Cell Precursors in Midgestational Embryonic Tissues

Following cultivation of disaggregated placental- or nonplacental embryonic tissue (NPET)-derived cells in 0.3 percent w/v agar, colonies were observed at the time of the first feeding (one week in culture) and at each subsequent weekly feeding. No attempt was made to quantitate the number of mast cell colonies prior to three weeks in culture because previous experience with liquid culture-derived mast cells indicated that early cultures were heterogeneous in morphology and cell surface phenotype (CHAPTER III). Reports of other cell lineages in similar semisolid cultures of less than three weeks duration confirmed our observations (Nakahata et al., 1982b; Pharr et al., 1984). All observations were therefore made between twenty-one and twenty-eight days of culture. Mast cell colonies, which predominate in long-term cultures, were identified by distinctive colony and cell morphology (Figure IV-1A, -1B). Non-mast cell colonies, consisting

Figure IV-1. Colonies in Long-Term Agar Cultures of Embryonic Cells in Conditioned Media.

Colonies of (BALB/c x CBA)F1 placental cells (day 12) were photographed at constant magnification after four weeks of growth in 0.3% agar with 50% W3CM.
(A) Small to medium mast cell colony;
(B) large mast cell colony;
(C) adherent, non-mast cells;
(D) diffuse non-mast cells;
(E) small granulocyte colony;
(F) mast cells were picked from the colony in A and stained with toluidine blue to visualize metachromatic granules.


A
B
C
D
E
F

of several distinguishable cell types (Figure IV-1C, -1D, -1E) were not enumerated in these studies. In addition to the colonial populations, long-term agar cultures also contained a large number of adherent cells. Although the identity of mast cells was not verified for each colony counted, staining of cells (picked from agar) with toluidine blue indicated that more than ninety percent of the colonies enumerated contained cells with metachromatic granules indicative of mast cells (Figure IV-1F).

Cells for culture were prepared from embryonic tissues of the two F1 crosses (BALB/cAN x CBA/J and BALB/cAN x B10.BR/SgSn) used in the Abelson murine leukemia virus infection experiments and from BALB/cAN homozygous embryos. As seen in Figure IV-2, the number of mast cell precursors in embryonic tissues is greatest in midgestation, peaking at eleven or twelve days post coitum and following the same distribution in both placenta and nonplacental embryonic tissues. This tendency was most evident in the heterozygous tissues, notably (BALB/cAN x CBA/J)F1, in which the number of mast cell colonies per one million inoculating cells increase twenty- to thirty-fold between days eight and twelve post coitum (Figure IV-2A). The increase in mast cell precursors during midgestation was less pronounced, but still significant, in the cells derived from (BALB/cAN x B10.BR/SgSn)F1 embryonic tissues, peaking between days ten and twelve with a ten- to twenty-

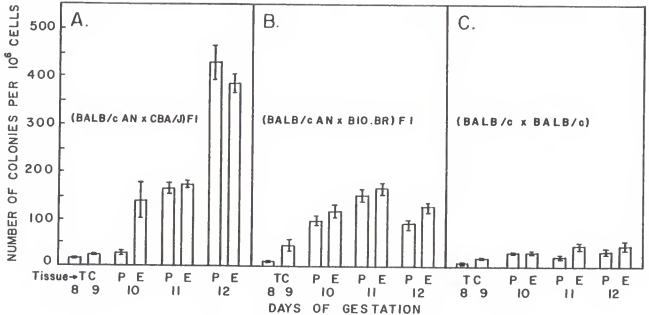


Figure IV-2. Frequency of Mast Cell Precursors in Midgestation Embryonic Tissues

Bars represent weighted means of 2 to 11 samples; error bars represent weighted standard deviations. Tissues analyzed: TC: total conceptus; P: placenta; E: nonplacental embryonic tissues.

fold increase over the levels observed at day eight (Figure IV-2B). In cells derived from homozygous tissues, however, the tendency toward increased numbers of mast cell precursors as embryonic development proceeded was much more subtle. Although the numbers of embryonic precursors were similar to those observed in heterozygous crosses earlier in gestation (day eight), the number of mast cell colonies in BALB/cAN tissues increased only three- to five-fold (Figure IV-2C).

Frequency of Mast Cell Precursors in Embryonic Tissues of the Third Trimester of Gestation

The increase in mast cell precursors in midgestation embryonic tissues, most dramatically demonstrated in the (BALB/cAN x CBA/J)F1 crosses, prompted us to ask whether the numbers were maintained in the placenta throughout the remaining course of gestation. As seen in Figure IV-3, the number of embryonic mast cell precursors in the placenta decreased seven- to eight-fold between days twelve and thirteen and then continued to fall rapidly to the lowest levels observed in our experiments by day fifteen.

Frequency of Mast Cell Precursors in Adult Bone Marrow

The number of mast cell precursors in the bone marrow of adult mice has been analyzed by a number of other investigators, thus providing a methodological control and standard to which the frequency of mast cells in embryonic tissue could be compared. It was additionally of interest

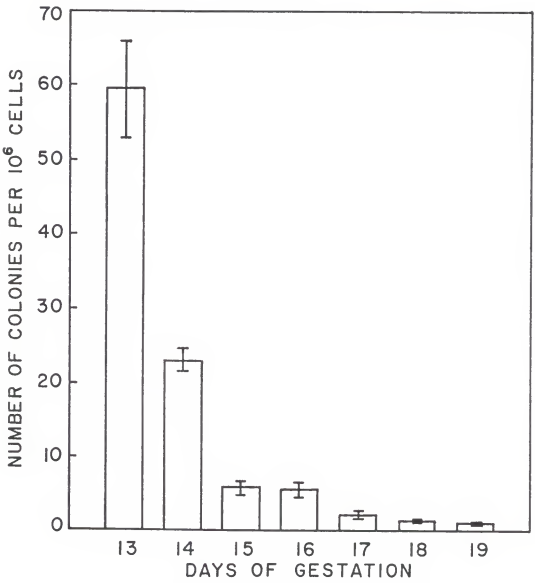


Figure IV-3. Frequency of Placental Mast Cell Precursors in the Third Trimester of Gestation.

Mast cells were cultured from (BALB/cAN x CBA/J)F1 placenta as indicated in Materials and Methods. Bars represent weighted means (Bahm,1972) of 5 to 13 samples; error bars represent standard deviations.

to determine whether the elevated number of mast cell precursors observed in heterozygous midgestation embryonic tissues, when compared to homozygous embryonic tissues, was maintained in the adult. As seen in Table IV-1, the number of precursors in BALB/cAN bone marrow was determined to be one per 2500 nucleated cells. These numbers are similar to those previously reported for other agar assays (Schrader et al., 1981; Sredni et al., 1983), as well as methylcellulose assays (Nakahata et al., 1982b; Pharr et al., 1984; Suda et al., 1985), and limiting dilution assays (Crapper and Schrader, 1983; Guy-Grand et al., 1984). In addition to the bone marrow data, we found that adult mouse spleen contained 30 mast cell precursors per million input cells, again consistent with previously published results (Nakahata et al., 1982b; Crapper and Schrader, 1983; Guy-Grand et al., 1984; Pharr et al., 1984).

Analysis of bone marrow derived from both of the heterozygous crosses demonstrated that these tissues are equally rich reservoirs of mast cell precursors. No quantitative differences in mast cell precursor frequency in (BALB/cAN x CBA/J)F1 and (BALB/cAN x B10.BR/SgSn)F1 bone marrows were found, nor was there a tendency toward elevated precursor numbers in the heterozygotes when compared to homozygotes. The differences in precursor

Table IV-1. Frequency of Mast Cell Precursors in Adult Bone Marrow from Homozygous and Heterozygous Mice

Strain	Number of Colonies per Million Bone Marrow Cells[a]				
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	All Experiments
BALB/cAN	450+/-150(2)	378+/-25.4(4)	371+/-20.9(3)		391+/-51.6(9)
B10.BR/SgSn	352+/-34.7(3)	308+/-8.5(3)	290+/-10(2)		320+/-18.7(8)
CBA/J	435+/-18.7(3)	398+/-13.1(3)	408+/-16.5(3)	420+/-8.2(3)	415+/-14.1(12)
(BALB/c x B10.BR)F1	301+/-13.4(4)	304+/-28.1(4)	377+/-28.7(3)	382+/-6.2(3)	336+/-19.3(14)
(BALB/c x CBA)F1	338+/-22.8(4)	350+/-36(4)	403+/-20.5(3)	402+/-19.3(3)	369+/-25.3(14)

a: Mast cell cultures in agar were prepared as described in Materials and Methods. Numbers represent statistical means +/- one standard deviation (number of cultures). Data for all experiments were calculated as weighted means and standard deviations (Bahn,1972).

frequency observed between heterozygous and homozygous tissues, therefore, are only limited temporally to a short period during midgestation.

Analysis of Surface Markers and Metachromasia of Colony Cells

Mast cell colonies are the predominant colony type between three and four weeks of in vitro growth in agar; other cell types, however, do persist in the presence of WEHI-3 conditioned media. We have frequently observed partially or confluent monolayers of adherent cells, sometimes associated with colonies of mast cells, as seen in Figure IV-1C. Also evident are colonies of cells both larger and smaller than mast cells which have distinctive morphologies (Figure IV-1D, -1E). The lineages of the non-mast cell types were not investigated in these studies, as they have been extensively described in the past (Nakahata et al., 1982b; Pharr et al., 1984).

Although it would be technically impossible to verify the identity of the cells comprising each enumerated mast cell colony, we picked a number of representative colonies to that end. The presence of metachromatic granules is a hallmark of mast cells of both the connective tissue and mucosal/cultured mast cell types (Jarrett and Haig, 1984). Macroscopic colonies of mast cells were identified initially at 100X magnification on an inverted, phase contrast microscope (Swift Instruments International,

Tokyo, Japan) and were picked under sterile conditions by gentle aspiration with a Pasteur pipet. The colonies were expelled from the pipet into a well of a 96-well tissue culture cluster (Costar, Broadway, MA) and the cells were disaggregated by gentle trituration with 0.1 ml of 50% W3CM. Some cells were analyzed immediately, while others were incubated in a humidified atmosphere of five percent carbon dioxide in air for one to two days prior to analysis. Approximately half of the cells were prepared for toluidine blue staining by cytocentrifugation and air drying. Following fixation in Mota's fixative (Yam et al., 1971), or acidic ethanol (Mota's fixative without lead subacetate), the slides were stained with 0.1 percent w/v toluidine blue, ph 0.5. Most (at least 90%) of the cells from each colony contained metachromatic granules indicative of mast cells (Figure IV-1F, Table IV-2).

Similarly identified and picked cells were also analyzed for surface markers as previously described. The cells had receptors for IgE but not for IgG, further confirming their identity as mast cells. Most of the cells also expressed the surface determinant recognized by monoclonal antibody B23.1. As previously noted, the latter marker is expressed by both mast cells and mononuclear phagocytes.

The expression of the B220 lymphoid marker recognized by the monoclonal antibody RA3-3A1 on A-MuLV-transformed

Table IV-2. Expression of Surface and Cytochemical Markers on Colony-Derived Mast Cells[a].

Colony Source	Percent cells expressing marker[b]			
	RA3-3A1	B23.1	IgE	Metachromatic granules
d10 embryo	67	84	56	100
d10 placenta	55	65	48	89
d11 embryo	36	83	51	100
d11 placenta	47	78	53	96
d12 placenta	37	89	55	99
bone marrow	43	94	52	94
bone marrow	47	91	54	97

[a] cells were cultured in 0.3% agar, 50% W3CM, for three to four weeks before analysis.

[b] cells were analyzed for cell surface markers and toluidine blue metachromasia as indicated in Materials and Methods.

placental cells prompted us to assay for its expression on colony-derived mast cells. Most of the colonies analyzed contained a significant number of cells which expressed the antigen recognized by the RA3-3A1 antibody (Table IV-2), in contrast to the results of analyses of numerous liquid culture-derived mast cells from similar sources (Chapter III).

Surface Markers on Mast Cell Precursors: Sorting of Cells with Monoclonal Antibodies

The presence of the determinants recognized by monoclonal antibodies B23.1 and RA3-3A1, and well as receptors for IgE, on the surface of both transformed and agar-cultured mast cells led us to ask whether these antigens were also expressed on the agar-colony mast cell precursors. Conditions for the sorting experiments were initially optimized on tumor cell lines previously shown to express high levels of the markers of interest. As seen in Table IV-3, biotinylated antibody plus avidin-modified sheep red blood treatment, followed by density gradient separation, was effective in depleting the control populations of most marker-positive cells. Rosetted cells, observed in suspension after staining nucleated cells with crystal violet (0.2 percent w/v), accounted for virtually all of the cells in the marker-positive, Lympholyte M pellets. On the other hand, few of the cells in the marker negative gradient supernatant were associated with SRBC in

Table IV-3. Sorting of Control Cells by Rosetting

Cell Line	Rosetting Agents	Phase of Separation Medium Analyzed	Percent Rosetted Cells: Crystal Violet Method[a]	Percent Rosetted Cells: Cyto-Centrifuge Method[b]
10P8	IgE/TNP-SRBC	supernatant	7	ND
		pellet	96	82
	PBS/TNP-SRBC	supernatant	0	ND
		pellet	0	0
11P62	B23.1-Biotin /Avidin-SRBC	supernatant	11	ND
		pellet	98	0
	PBS/Avidin-SRBC	supernatant	0	ND
		pellet	0	0
18-81	RA3-3A1-Biotin/ Avidin-SRBC	supernatant	8	ND
		pellet	100	95
	PBS/Avidin-SRBC	supernatant	0	ND
		pellet	0	0

a. Following sorting of cells, an aliquot was made 0.2% crystal violet and the number of rosetted and unrosetted cells per 10^{-4} ml hemocytometer field were counted on an inverted phase microscope.
b. Following sorting of cells, an aliquot was cyto-centrifuged and stained with Wright's Giemsa. Coverslips were affixed with Permount and at least one hundred cells were microscopically analyzed for association with three or more sheep red blood cells.

suspension. Cytocentrifuged samples of the preparations showed somewhat lower numbers of rosetted cells, presumably due to mechanical shearing and lysis of SRBC during the processing. The latter data, however, are similar to those generated by the S. aureus-antibody sandwich technique.

The data for the sorting of BALB/cAN bone marrow are summarized in Table IV-4. Several significant observations are evident. First, if bone marrow cells (unfractionated) were incubated on ice but not subjected to antibody treatment, rosetting, gradient separation, lysis and numerous centrifugations in the time-consuming (eight to twelve hour) procedure, the number of mast cell colonies was reduced approximately forty percent compared to previous, unsorted experiments. This decrement was presumably due to the extended processing time in the absence of growth factor, which was two to three times that required to do the initial experiments. Second, the manipulation of cells by incubation with antibody and sheep red blood cells, separation on a density gradient medium, incubation with ammonium chloride or hypotonic salts solutions, and numerous centrifugations resulted in no significant further depletion of mast cell precursors, as evident from the similar number of colonies in sorted and unsorted marrow populations. Finally, the method of lysis of SRBC did not significantly alter the results of the assay. This last observation is surprising in light of the

Table IV-4. Sorting of Bone Marrow Cells by Surface Determinants

Experiment[a]	Rosetting Agents[b]	Number of Colonies per 10^6 Cells Analyzed[c] Unfractionated Control[d]	Marker Positive[e]	Marker Negative[f]	Reconstructed Population
1A	B23.1-Biotin/ Avidin-SRBC	212+/-13(3)	185+/-23(3)	ND[g]	178+/-2(2)
1B	RA3-3A1-Biotin/ Avidin-SRBC	212 10(3)	ND	190+/-22(3)	185 5(2)
1C	IgE/TNP-SRBC	240 14(3)	ND	205 5(2)	148 8(2)
2A	IgE/TNP-SRBC	232 26(3)	2 2(3)	230 22(3)	175[h]
2B	B23.1-Biotin/ Avidin-SRBC	220 20(2)	222 20(2)	ND	215 15(2)
2C	RA3-3A1-Biotin/ Avidin-SRBC	ND	0(1)	215 55(2)	232 8(2)

a: Experiments 1A, 1B, 1C were performed with 3 to 6 x 10^6 cells prior to sorting and with ammonium chloride-mediated SRBC lysis. Experiments 2A, 2B, 2C were performed with 0.7 to 2.8 x 10^7 cells prior to sorting and with hypotonic shock-mediated SRBC lysis.

b: Sorting was performed with the antibody/SRBC combination as indicated in Materials and Methods.

c: Numbers represent mean number of colonies (standardized per 10^6 cells per well) +/- 1 standard deviation (number of wells analyzed).

d: Unfractionate controls were kept on ice without rosetting agents for the duration of the sorting experiment and plated in agar at the same time as the sorted cells.

e: The "marker positive" fraction consisted of sorted cells from the separation medium pellet which were subsequently depleted of sheep red blood cells.

f: The "marker negative" fraction consisted of sorted cells from the separation medium supernatant which were subsequently depleted of sheep red blood cells.

g: ND: experiment not done due to insufficient cells in the indicated fractions.

h: Only one well plated due to insufficient cells.

remaining TNP-SRBC in the cultures of IgE-sorted bone marrow cells; these cells apparently had no effect on the proliferation of mast cell precursors in agar.

Sorting of bone marrow with B23.1-biotin and avidin-modified SRBC resulted in the segregation of all of the mast cell precursors to the marker-positive fraction. Ninety to one hundred percent of the precursors were recovered from the rosetted pellet when compared to unsorted cells. Dilutions of sorted cells cultured in agar showed linearity of inoculum versus colony number, indicating that the assay was a reliable measure of precursor frequency. If cells from each fraction were combined in proportion to the number which partitioned with the rosettes (pellets) or free cells (supernatants), the frequency of colonies enumerated was the same as that in unfractionated bone marrow. This last observation indicated that the minute B23.1 marker-negative population contributes neither stimulatory nor inhibitory factors required for the efficient seeding of mast cell precursors.

When bone marrow cells were sorted with biotinylated-RA3-3A1 antibody and avidin-modified SRBC, all of the mast cell precursors were found in the marker-negative (supernatant) fraction. As seen with the B23.1 sorting, essentially all (ninety percent) of the mast cell precursors were recovered after RA3-3A1 sorting, and the plot of the cells seeded versus mast cell colonies was

linear in two experiments. The number of mast cell colonies observed after reconstitution of both fractions at the correct ratio was very close to the anticipated value in one experiment and slightly greater than (140 percent) the anticipated value in the second experiment, perhaps indicating synergism between the partitioned cell types in the formation of colonies. Further experiments to test the cooperative hypothesis would be required before the latter could be concluded.

Bone marrow sorted with IgE anti-TNP and TNP-SRBC exhibited partition characteristics similar to that sorted with RA3-3A1. Eighty-five to ninety-nine percent of the mast cell precursors were recovered following sorting in two independent experiments using two different methods to lyse the SRBC. Cells seeded versus colonies enumerated were linear in both experiments, and the number of colonies counted in reconstitution experiments was similar to those anticipated if only cells from the marker-negative (supernatant) population were seeded, indicating that the mast cell precursors found in the IgE receptor-negative fraction were independent of IgE receptor-positive cells in their ability to form mast cell colonies.

Discussion

The development of mast cells from embryonic tissues in liquid cultures led us to quantitate the number of mast cell precursors in those tissues. The frequency of such precursors has historically been analyzed by several methods. Nakahata et al. (1982b) developed a culture assay for mouse mast cell colonies in methylcellulose. The assay was adapted from previous work (Parmley et al., 1976) which enumerated multipotent hematopoietic progenitors, but selected for mast cell growth with lectin-stimulated conditioned media. Subsequent use of the technique by others in the same laboratory (Pharr et al., 1984; Suda et al., 1985) has established the reliabilty of this method. Concurrent to the development of the methlcellulose assay, Schrader et al. (1981) and Zucker-Franklin et al. (1981) reported the development of a similar clonal assay using agar-based semisolid media with results similar to those obtained with methylcellulose. In addition to the semisolid medium techniques, limiting dilution analysis in liquid culture has been used to quantitate the cultured mast cell precursor frequency in a variety of tissues (Crapper and Schrader, 1983; Guy-Grand et al, 1984).

Based on experience, supplies, and the similar results obtained in independent studies (above), we chose to analyze the frequency of mast cells in embryonic tissues by

a clonal agar assay (Schrader et al., 1981). We modified the assay, however, to be consistent with our previous liquid culture experiments (Chapter III), by substituting WEHI-3 condidtioned media (Razin et al., 1984a) for the Con A- spleen-conditioned media of those authors. We verified the reliability of our assay by quantitation of bone marrow and spleen-derived mast cell precursors and found that our data were consistent with those previously published.

The results of our analyses of mast cell precursors in midgestation and late gestation embryonic tissues present several significant observations. First, we have described the earliest mast cell precursors reported in the mouse, being first detected at eight days post coitum. Previous to this report, the earliest reported mast cell precursors were derived from disaggregated day ten or eleven embryos which had been cultured in the presence of T cell-derived growth factors (Ginsburg et al, 1982). In the mouse, embryonic mast cell precursors have also been identified in the fetal liver as early as day thirteen post coitum (Kitamura et al., 1979c; Nabel et al., 1981). Similar observations have been reported in the embryonic rat (Ishizaka et al., 1976) and in the embryonic human (Razin et al., 1981b).

A second significant observation concerns the frequency of embryonic mast cell precursors as gestation progresses. As seen in Figure IV-2, the number of mast

cell precursors increases between days eight and twelve. In the most dramatic case studied, this augmentation represented a twenty- to thirty-fold increase in mast cell colonies for (BALB/cAN x CBA/J)F1 embryos (reaching levels similar to those detected in adult bone marrow), while smaller increases were observed for (BALB/cAN x B10.BR)F1 and homozygous BALB/cAN embryos. The discrepancies between heterozygous and homozygous embryonic mast cell precursor frequencies were not observed in adult bone marrow, leading us to speculate that allogeneic differences may be responsible for mast cell precursor proliferation at this critical period of fetal development.

A third significant observation of this study is that the number of mast cell precursors in the murine embryonic placenta drops rapidly from its peak at twelve days to the lowest levels noted in that tissue during the course of the third trimester of pregnancy. Our observation that day eleven embryonic tissue contained fewer mast cell precursors than those of day thirteen were supported by similar reports in the literature (Ginsburg et al., 1982). Although precursor frequency in the embryo proper was not investigated during the same period of time, we have noted that Kitamura et al. (1979c) reported the isolation of mast cell precursors in the fetal liver of day thirteen and day fourteen mouse embryos; the former observation was substantiated by Nabel et al. (1981).

We propose that mast cell precursors in the midgestation placenta either perish (due to deprivation of interleukin 3 in their microenvironment or programmed annihilation) or migrate around day thirteen to a new microenvironment in the fetal liver, mucosal sites, or perhaps the thymus. The fetal liver is the major hematopoietic organ of late gestation (Metcalf and Moore, 1971). In support of the model of mast cell precursor annihilation in the third trimester placenta is a recent report of "waves" of pluripotent hematopoietic stem cells (CFU-S) which respond to interleukin 3 (Spivak et al., 1985). Like mast cells, CFU-S require interleukin 3 for survival as well as proliferation in vitro; in the absence of interleukin 3, CFU-S concentration decreased precipitously. Even in the presence of interleukin 3, the number of proliferating cells exhibited cyclic fluctuations, which may be an inherent property of all hematopoietic cells (King-Smith and Morley, 1970). If such processes were in force in vivo as well as in vitro, we would propose the loss of interleukin 3 responsiveness in placenta-associated mast cell precursors beginning at day thirteen (following at least three days of extensive proliferation) and the concurrent increase in responsiveness of fetal liver-associated mast cell precursors (Kitamura et al., 1981) represent these waves.

A fourth significant observation of this work concerns the expression of mast cell-specific markers on bone marrow-derived cells picked from colonies between three and four weeks in culture. Initial attempts to propagate mouse mast cells in agar were met with complete failure (McCarthy et al., 1980). Colonies, which were observed after one week in culture, yielded no cells with astra blue-staining granules characteristic of mast cells. Later investigators (Schrader et al., 1981; Zucker-Franklin, 1981; Nakahata et al., 1982b) established that a minimum of two weeks in culture was required for mouse mast cell maturation. Longer incubation periods were, in fact, preferable, since mast cell colonies are less transient than those composed of other lineages (Pharr et al., 1984). The presence of mast cells in macroscopic colonies was first confirmed in situ by microscopic observation of round cells with clearly delineated, refractile edges and slight dark hue (Nakahata et al., 1982b). More than ninety percent of the cells picked from such colonies contained metachromatic granules when stained with acidic toluidine blue. These cells also had surface receptors for IgE (but not IgG) and most expressed the surface determinant recognized by the monoclonal antibody B23.1, and thus, by three criteria, could be identified as mast cells.

The colony-derived mast cells also expressed the B-lineage variant of the Ly5 surface antigen. Ly5 has

previously been observed on cultured mast cells (Nabel et al., 1981; Wong et al., 1982) and mast cell tumors (Scheid and Triglia, 1979); in addition, it was detected on two A-MuLV-transformed placental mast cell lines and on the murine mastocytoma P815 and two A-MuLV/pristane-induced mastocytomas (Siegel et al., 1985; Chapter II). The expression of Ly5 on the surface of mast cells may represent a discreet stage in the maturation which is not attained under the conditions of our liquid culture system (Chapter III).

The final aspect of our studies described in this chapter dealt with the determination of cell surface markers on mouse bone marrow-derived mast cell precursors. Although the literature contains numerous references to surface markers on liquid culture-derived mast cells (reviewed by Katz et al., 1985a), we were only able to find a single reference to expression of such markers on mast cell precursors (Yung et al., 1983). Treatment of mouse bone marrow cells with anti-Ia and rabbit complement resulted in the loss of fifty percent of the interleukin 3-responsive (proliferating) population; the same treatment also depleted granulocyte-macrophage colony-forming units (CFU-GM) by the same factor.

Three surface determinants detected on A-MuLV-transformed embryonic mast-like cell lines were selected for sorting mast cell precursors. The sorting methodology

was optimized and proven effective on previously characterized mouse cell lines (Table IV-3). The time-consuming technique in the loss of approximately forty percent of the mast cell precursors, presumably due to the extended processing time in the absence of interleukin 3 and not numerous manipulations. By combining the sorting procedure with the cultivation of cells in semisolid agar media, we were able to effectively deplete all of the mast cell precursors with B23.1 antibody; in contrast, neither IgE nor RA3-3A1 significantly depleted bone marrow of mast cell precursors. These preliminary results, however, were questionable in light of previous experience with the same antibodies in the system used to detect surface markers on liquid culture cell populations (see Chapter III). Under the latter conditions, fresh bone marrow contained twenty two percent RA3-3A1 reactive cells and six percent B23.1 positive cells. In the sorting procedure, bone marrow contained three to twenty-four percent RA3-3A1 positive cells and ninety-six to ninety-nine percent B23.1 positive cells. The reason for this discrepancy is unknown, since all experimental controls gave predicted values. Despite the intertechnique inconsistencies, we are confident that few or no mast cell precursors express receptors for IgE or the determinant recognized by monoclonal antibody RA3-3A1, while it would appear that most, if not all, may express the determinant recognized by B23.1. Further studies, with

efforts to minimize nonspecific depletion of cell populations, will be required to better define the surface phenotype of precursors to cultured mast cells.

The midgestation murine conceptus is the site of a number of interesting and perhaps time-related phenomena which may be associated with the presence of culture-derived mast cell precursors. The pluripotent hematopoietic stem cell, which gives rise to all of the hematopoietic lineages and is quantitated in the in vivo CFU-S assay (Till and McCulloch, 1961), has been isolated during embryogenisis from the blood islands of the yolk sac between days eight and ten and from the fetal liver throughout the remainder of gestation (Moore and Metcalf, 1970). Mast cells have been shown to arise from CFU-S (Kitamura et al., 1981) as well as multipotent in vitro colony forming cells (Schrader et al., 1981; Sonoda et al., 1983; Pharr et al., 1984). Furthermore, CFU-S and their in vitro correlates are responsive to the proliferative effects of interleukin 3 (Goldwasser et al., 1983; Garland and Crompton, 1983; Spivak et al., 1985; Rennick et al., 1985), the mast cell growth factor prevalent in WEHI-3 conditioned media. The presence of mast cell precursors in embryonic tissues as early as eight days of gestation may thus be an indication of multipotent hematopoietic stem cells or their interleukin 3-responsive progeny which differentiate into mast cells in the continued presence of

that growth factor. The absence of mature mast cells from the same tissues may be indicative of *in vivo* control mechanisms which are abrogated by *in vitro* propagation.

Abelson murine leukemia virus targets are most frequent in embryonic tissues at day ten of gestation, while the number of culture-derived mast cell precursors peaks at day twelve. A-MuLV is capable of transforming cells of most of the hematopoietic lineages, among them mast cells (Chapter III; Pierce et al., 1985). We propose that the cells detected in the transformation assay are precursors to the untransformed cells which proliferate in response to interleukin 3. The difference between the two populations may be one of yet undescribed differentiation markers: Cells which are targets for A-MuLV, not yet responsive to interleukin 3, may further differentiate into cells which are responsive to interleukin 3 but no longer possess receptors for the transforming virus. This model remains untested and its mechanisms demand further elucidation. We have previously proposed that the A-MuLV oncogene product may substitute for interleukin 3, thus inducing the mast cell-like characteristics of our transformed placental cell lines (Siegel et al., 1985). The expression of c-*abl* messenger RNA, which also peaks in the embryo at ten days of gestation (Muller et al., 1982) and may encode a growth factor activity which normally acts

on the virus transformation-sensitive target cells which may then differentiate into interleukin 3- responsive culture-derived mast cell precursors.

The studies described in this chapter may also contribute to the understanding of the immunobiology of the maternal-fetal interface. The allogeneically foreign conceptus first presents immunogenic antigens from the cytotrophoblast to its mother between days nine and eleven of gestation (Roe and Bell, 1982). The spectrum of maternal-fetal immunological reactions have been extensively reviewed in the literature (Bell and Billington, 1983; Chaouat et al., 1983; Lala et al., 1983). The mother, recognizing the fetal graft as nonself, responds with both humoral and cell-mediated arms of the immune system. The placenta, which has been described as an immunological barrier and an immunoadsorbent (Wegmann et al., 1979), also has been reported to play an active immunoregulatory role (Chaouat et al., 1980; Remacle-Bonnet et al., 1983; Chaouat and Kolb, 1985), although the cellular source(s) of immunomodulatory factors is not defined. Histamine, which is reported to play an important role in blastocyst implantation (Dey et al., 1979; Dey and Johnson, 1980a, 1980b; Nalbandov, 1971), has also been shown to inhibit cytotoxic T lymphocyte effector functions (Plaut et al., 1973; Schwartz et al., 1980; Chaouat and Kolb, 1985), to inhibit the production of macrophage

inhibitory factor (Rocklin, 1976) and to have other immunomodulatory effects (Askenase et al., 1981) as well as promoting tissue growth and repair (Kahlson and Rosengren, 1968). Prostaglandins and leukotrienes, related mast cell products, may play similar immunomodulatory roles (Lala et al., 1983). Although our studies of freshly dissociated tissues have indicated that mast cells are, at most, a minor fraction of the total conceptus between days ten and twelve post coitum, a small but significant population of cells at the maternal-fetal interface could indeed contribute to the maintenance of the fetal graft.

CHAPTER V
SUMMARY AND CONCLUSIONS

The experimental procedures described in the preceding pages have enabled us to report a number of novel and significant observations which contribute to the existing body of knowledge concerning mast cells. We began our studies following the observation of cells with basophilic and metachromatic granules in lines of Abelson murine leukemia virus-transformed cells which were derived from in vitro-infected midgestation mouse embryonic placentae. The relationship of the A-MuLV-transformed cells to culture-derived mast cells was further substantiated by the observation that both expressed the epitope recognized by the B23.1 monoclonal antibody, which binds to a determinant on culture-derived, but not peritoneal, mouse mast cells. Most of the cell lines also contained histamine in quantities similar to those found in culture-derived mast cells. Furthermore, there was a direct correlation between the histamine content and the expression of surface IgE receptors, another mast cell phenotypic marker, in the cell lines which were analyzed. In contrast to culture-derived mast cells, however, the A-MuLV-transformed mast-like cells proliferated in the absence of exogenous interleukin 3. Unlike other autogenous mast-like cells (Schrader and Crapper, 1983) but similar to recently reported A-MuLV fetal liver transformants (Pierce et al., 1985), the cell lines produced no detectable interleukin 3.

In the course of characterizing the Abelson virus-transformed embryonic cell lines, we developed a sensitive, nonisotopic, nonfluorometric assay for membrane receptors for cytophillic immunoglobulin. The method utilizes hapten-specific monoclonal antibodies and homologous hapten-derivatized bacteria which form rosettes with cells bearing the appropriate receptors. The marker positive cells are easily identified by light microscopy of cytocentrifuged, fixed, and stained preparations. The technique has the advantage over previously described isotopic (Mendoza and Metzger, 1976) and nonisotopic (Schrader, 1981) methods of allowing morphological characterization of the reactive cells. This methodology is applicable to the characterization of other hematopoietic lineages and can be modified to identify two specificities on the same cell (Siden and Siegel, 1986).

The identification of mast cell characteristics in lines derived by the transformation of midgestation enbryonic placenta by Abelson murine leukemia virus led us to analyze homologous, untransformed embryonic tissues for mast cells and their precursors. Similar to the observations of Kitamura and colleagues (1979c) on mouse fetal liver as early as day thirteen post coitum, we detected no mast cells in embryonic tissues at days ten and eleven of gestation. We were, however, able to culture mast cells from those tissues, thus providing the novel

observation that mast cell precursors exist in the mouse embryo at least five days before the first mast cells are detectable (Kitamura et al., 1979c). Subsequent analysis of mast cell precursors in embryonic tissues between days eight and nineteen of gestation indicated that the precursors to culture-derived mast cells exist in very low numbers prior to day nine of gestation, increasing by day twelve to levels similar to those found in adult bone marrow. The number of embryonic placental precursors to culture-derived mast cells then falls precipitously between days thirteen and nineteen of gestation. The results are particularly interesting in light of the previously noted observation that in vivo mast cell precursors are abundant in the fetal liver at day thirteen (Kitamura et al., 1979c). It is enticing to speculate that mast cell precursors either migrate through the placenta to the fetal liver between days twelve and thirteen, or an independent second "wave" of precursors develops in the fetal liver at that time; however, there is no definitive evidence that the embryonic in vitro and in vivo mast cell precursors are identical.

In the course of our investigations, we studied the progression of five hematopoietic markers in mast cell cultures of embryonic and adult tissues over the course of four weeks. Previous reports of phenotypic changes in mast cell cultures were limited to morphological and

cytochemical analysis performed at the microscopic level, with surface determinant characterization performed on mature culture-derived mast cells. In contrast, our investigations documented the sequential selection and enrichment of cells expressing both membrane receptors for IgE and the culture-derived mast cell determinant recognized by the monoclonal antibody B23.1 (Katz et al., 1983), as well as metachromatic granules, from a heterogeneous population of cells relatively impoverished of the markers. We feel that our observations complement those of Ginsburg (1963) and later investigators and add a further level of analytical sophistication to their historical contributions.

Two features of the Abelson virus-transformed mast cell-like lines, interleukin 3 independence and expression of the B lineage variant of the Ly 5 differentiation marker, were notably absent from cultured mast cells derived from the same tissues. Culture-derived mast cells were infected with Abelson virus to investigate the role of that agent in the induction of those characteristics. The virus-infected cells exhibited factor-independent growth, but were still devoid of the lymphoid marker. Observation of nonadherent cells expressing the same lymphoid determinant in cultures of unselected (adherent and nonadherent) uninfected cells grown in the presence of interleukin 3 prompted us to investigate the role of

adherent cells and their factors on culture-derived mast cells. Long-term culture-derived mast cells did not express the B lineage variant of Ly 5 before or after coculture with adherent cells of several sources. We have therefore concluded that neither Abelson murine leukemia virus nor adherent cell monolayers were responsible for the expression of a B lymphoid determinant on the mast-like A-MuLV-transformed cell lines.

In the final chapter of this dissertation, we enumerated and characterized mast cell precursors in embryonic tissues and in adult bone marrow. Mast cells derived from semisolid agar cultures expressed the B lymphoid Ly 5 variant previously noted on the surfaces of A-MuLV-transformed mast-like cells. Based on these and previous observations, we have proposed that the expression of such surface markers on agar culture-derived mast cells represents a discreet stage of mast cell differentiation which is not observed in liquid culture.

The ability to ascribe surface determinants to cultured mast cell precursors may provide us with a better understanding of the differentiation of all mast cells. We have sorted primary bone marrow cells with monoclonal antibodies which recognize surface determinants previously noted on culture-derived mast cells and on A-MuLV-transformed mast-like cells. Cells lacking the B lymphoid variant of Ly 5 and receptors for IgE were

observed to provide a rich source of mast cell precursors, while cells expressing the same markers produced few mast cell colonies in semisolid agar. In contrast, cells rosetted by red blood cells bearing monoclonal antibody B23.1 were as rich in mast cell precursors as untreated cells. These preliminary studies have significantly contributed to the meager body of information which describes the phenotype of the culture-derived mast cell precursor (Yung et al., 1983).

In conclusion, the investigations presented in this dissertation have demonstrated the existence of culture-derived mast cell precursors in the earliest reported stages of murine development. Although its role in hematopoiesis is yet undefined, the embryonic mouse placenta is a rich source of culture-derived mast cell precursors for a brief period of fetal development. These findings may serve as an impetus for further studies which will better define the role of interleukin 3-responsive cells in embryogenesis and mast cell differentiation.

REFERENCES

Abelson, H.T., and L.S. Rabstein. 1970. Lymphosarcoma: Virus-induced thymic-independent disease in mice. Cancer Res. 30: 2213-2222.

Aldenborg, F., and L. Enerback. 1985. Thymus dependence of connective tissue mast cells: A quantitative cytofluorometric study of the growth of peritoneal mast cells in normal and athymic mice. Int. Archs. Allergy Appl. Immunol. 78: 277-282.

Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore. 1981. Organization and reorganization of immunoglobulin genes in A-MuLV transformed cells: Rearrangement of heavy but not light chain genes. Cell 27: 381-390.

Alt, F.W., G.D. Yancopoulos, T.K. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore. 1984. Ordered rearrangement of immunoglobulin heavy chain variable region segments. The EMBO Journal 3: 1209-1219.

Asboe-Hansen, G. 1971. Mast cells and the skin. In E.B. Helwig and F.K. Mostofi (eds.). The Skin. Williams and Wilkins, Baltimore, MD.

Askenase, P.W. 1977. Role of basophils, mast cells, and vasoamines in hypersensitivity reactions with a delayed time course. Prog. Allergy 23: 199-320.

Askenase, P.W. 1980. Immunopathology of parasitic diseases: Involvement of basophils and mast cells. Springer Semin. Immunopathol. 2: 417-442.

Askenase, P.W., A. Schwartz, J.N. Siegel, and R.K. Gershon. 1981. Role of histamine in the regulation of cell-mediated immunity. Int. Archs. Allergy Appl. Immunol. 66 (Suppl. 1): 225-233.

Austen, K.F. 1984. The heterogeneity of mast cell populations and products. Hosp. Pract. 19: 135-146.

Bahn, A.K. 1972. Basic Medical Statistics. Grune-Stratton, New York, NY.

Ball,P.E., M.L. Conroy, C.H. Heusser, J.M. Davis, and J.F. Conscience. 1983. Spontaneous, in vitro, malignant transformation of a basophil/mast cell line. Differentiation 24: 74-78.

Baltimore, D., N. Rosenberg, and O.N. Witte. 1979. Transformation of immature lymphoid cells by Abelson murine leukemia virus. Immunol. Rev. 48: 3-22.

Bazill, G.W., M. Haynes, J. Garland, and T.M. Dexter. 1983. Characterization and partial purification of a haemopoietic cell growth factor in WEHI-3 cell conditioned medium. Biochem. J. 210: 747-759.

Befus, A.D., and J. Bienenstock. 1979. Immunologically mediated intestinal mastocytosis in Nippostrongylus brasiliensis-infected rats. Immunology 38: 95-101.

Befus, A.D., F.L. Pearce, J. Gauldie, P. Horsewood, and J. Bienenstock. 1982a. Mucosal mast cells. I. Isolation and functional characteristics of rat intestinal mast cells. J. Immunol. 128: 2475-2480.

Befus, A.D., F.L. Pearce, G. Goodacre, and J. Bienenstock. 1982b. Unique functional characteristics of mucosal mast cells. Adv. Exp. Med. Biol. 149: 521-527.

Bell, S.C., and W.D. Billington. 1983. Anti-fetal allo-antibody in the pregnant female. Immunol. Rev. 75: 5-30.

Benditt, E.P., S. Bader, M. Arase, C. Corley, and K.B. Lam. 1954. Relationship of mast cells and histamine to mechanism of edema production by ovomucoid in rats. Fed. Proc. 13: 422-423.

Benveniste, et al. 1972. A possible role for IgE in immune complex disease. In The biological role of the immunoglobulin E system. K. Ishizaka and D.H. Dayton, Jr. (eds). U.S. Government Printing Office.

Bienenstock, J. 1984. The mucosal immunologic network. Ann. Allergy 53: 535-540.

Bienenstock, J., A.D. Befus, F. Pearce, J. Denburg, and R. Goodacre. 1982. Mast cell heterogeneity: Derivation and function with emphasis on the intestine. J. Allergy Clin. Immunol. 70: 407-412.

Bienenstock, J., A.D. Befus, M. McDermott, S.Mirski, K. Rosenthal, and A. Tagliabue. 1983. The mucosal immunological network: Compartmentalization of lymphocytes, natural killer cells, and mast cells. Ann. N.Y. Acad. Sci. 409: 164-170.

Boss, M., M. Greaves, and N. Teich. 1979. Abelson virus-transformed haematopoietic cell lines with pre-B-cell characteristics. Nature 278: 551-553.

Brandon, J.M., and M.C. Bibby. 1979. A study of changes in uterine mast cells during early pregnancy in the rat. Biol. Reprod. 20: 977-980.

Burgess, A.W., D. Metcalf, S.H.M. Russell, and N.A. Nicola. 1980. Granulocyte/macrophage-, megakaryocyte-, eosinophil-, and erythroid-colony stimulating factors produced by mouse spleen cells. Biochem J. 185: 301-314.

Burnet, F.M. 1965. Mast cells in the thymus of NZB mice. J. Path. Bact. 89: 271-284.

Burnet, F.M. 1975. Possible identification of mast cells as specialised post-mitotic cells. Med. Hypoth. 1: 3-5.

Burnet, F.M. 1977. The probable relationship of some or all mast cells to the T-cell system. Cell Immunol. 30: 358-360.

Burton, A.L. 1963. Studies on living normal mast cells. Ann. N.Y. Acad. Sci. 103: 245-263.

Capron, M., J. Rousseaux, C. Mazingue, H. Bazin, and A. Capron. 1978. Rat mast cell-eosinophil interaction in antibody-dependent eosinophil cytotoxicity to Schistosoma mansoni schistosomula. J. Immunol. 121: 2518-2525.

Caulfield, J.P., R.A. Lewis, A. Hein, and K.F. Austen. 1980. Secretion in dissociated human pulmonary mast cells. J. Cell. Biol. 85: 299-311.

Chaouat, G., S. Chaffaux, M. Duchet-Suchaux, and G.A. Voisin. 1980. Immunoactive products of the mouse placenta. I. Immunosuppressive effects of crude and water-soluble extracts. J. Reprod. Immunol. 2: 127-139.

Chaouat, G., and J.-P. Kolb. 1985. Immunoactive products of the placenta. IV. Impairment by placental cells and their products of CTL function at effector stage. J. Immunol. 135: 215-222.

Chaouat, G., J.P. Kolb, and T.G. Wegmann. 1983. The murine placenta as an immunological barrier between the mother and the fetus. Immunol. Rev. 75: 31-60.

Clark-Lewis, I., R.M. Crapper, K. Leslie, S. Schrader, and J.W. Schrader. 1985. P cell stimulating factor; molecular and biological properties. In C. Song and A. Schimpl (eds.). Cellular and Molecular Biology of Lymphokines. Academic Press, Orlando, FL.

Clark-Lewis, I., S.B.H. Kent, and J.W. Schrader. 1984. Purification to apparent homogeneity of a factor stimulating the growth of multiple lineages of hemopoietic cells. J. Biol. Chem. 259: 7488-7494.

Clark-Lewis, I., and J.W. Schrader. 1981. P cell-stimulating factor: Biochemical characterization of a new T cell-derived factor. J. Immunol. 127: 1941-1947.

Clark-Lewis, I., J.W. Schrader, Y.-Y. Wu, and A.W. Harris. 1982. A B lymphoma cell line produces growth factors for hemopoietic, lymphoid, and mast cells. Cell Immunol. 69: 196-200.

Cobb, C.M., H. Birkedal-Hansen, and F.R. Denys. 1975. Ultrastructural characteristics of mast cells from a canine mastocytoma maintained in vitro. J. Oral Pathol. 4: 244-256.

Code, C.F. 1977. Reflections on histamine gastric secretion and the H2 receptor. New Engl. J. Med. 296: 1459-1462.

Coffman, R.L., and I.L. Weissman. 1981a. A monoclonal antibody that recognizes B cells and B cell precursors in mice. J. Exp. Med. 153: 269-279.

Coffman, R.L., and I.L. Weissman. 1981b. B220: A B cell-specific member of the T200 glycoprotein family. Nature 289: 681-683.

Combs, J.W., D. Lagunoff, and E.P. Benditt. 1965. Differentiation and proliferation of embryonic mast cells of the rat. J. Cell. Biol. 25: 577-592.

Conscience, J.-F., and F. Fisher. 1985. Growth-factor-independent proliferation in vitro and tumorigenicity in vivo are associated in basophil/mast-cell lines and their somatic hybrids. Differentiation 28:291-295.

Cook, W.D. 1982. Rapid thymomas induced by Abelson murine leukemia virus. Proc. Natl. Acad. Sci. USA 79: 2917-2921.

Cook, W.D., D. Metcalf, N.A. Nicola, A.W. Burgess, and F. Walker. 1985. Malignant transformation of a growth factor-dependent myeloid cell line by Abelson virus without evidence of an autocrine mechanism. Cell 41: 677-683.

Cooper, J., and T. Hunter. 1981. Four different classes of retroviruses induce phosphorylation of tyrosine present in similar cellular proteins. Mol. Cell. Biol. 1: 394-407.

Crapper, R.M., and J.W. Schrader. 1983. Frequency of mast cell precursors in normal tissues determined by an in vitro assay: Antigen induces parallel increases in the frequency of P cell precursors and mast cells. J. Immunol. 131: 923-928.

Crowle, P.K. 1982. Bone marrow origin of mucosal mast cells. Fed. Proc. 41: 581.

Crowle, P.K., and N.D. Reed. 1984. Bone marrow origin of mucosal mast cells. Int. Archs. Allergy Appl. Immunol. 73: 242-247.

Csaba, G., and E. Kapa. 1960. Uptake of heparin by cells. Nature 187: 711-713.

Czarnetzki, B.M., and H. Behrendt. 1981. Studies on the in vitro development of rat peritoneal mast cells. Immunobiology 159: 256-268.

Czarnetzki, B.M., C.G. Figdor, G. Kolde, T. Vroom, R. Aalberse and J.E. de Vries. 1984. Development of human connective tissue mast cells from purified blood monocytes. Immunology 51: 549-554.

Czarnetzki, B.M., D. Hannich, and H. Niedorf. 1979. In-vitro studies on the development of rat peritoneal mast cells. Immunobiology 156: 470-476.

Czarnetzki, B.M., G. Kruger, and W. Sterry. 1983. In vitro generation of mast cell-like cells from human peripheral mononuclear phagocytes. Int. Archs. Allergy Appl. Immunol. 71: 161-167.

Czarnetzki, B.M., W. Sterry, H. Bazin, and K.J. Kalveram. 1982. Evidence that the tissue mast cells derive from mononuclear phagocytes. Int. Archs. Allergy Appl. Immunol. 67: 44-48.

Daeron, M., A.R. Sterk, F. Hirata, and T. Ishizaka. 1982. Biochemical analysis of glucocorticoid-induced inhibition of IgE-mediated histamine release from mouse mast cells. J. Immunol. 129: 1212-1218.

Davidson, S., A. Mansour, R. Gallily, M. Smolarski, M. Rofolovitch, and H. Ginsburg. 1983. Mast cell differentiation depends on T cells and granule synthesis on fibroblasts. Immunology 48: 439-452.

Denburg, J.A., A.D. Befus, and J. Bienenstock. 1980a. Growth and differentiation in vitro of mast cells from mesenteric lymph nodes of Nippostrongylus brasiliensis-infected rats. Immunology 41: 195-202.

Denburg, J.A., M. Davison, and J. Bienenstock. 1980b. Basophil production. J. Clin. Invest. 65: 390-399.

Denburg, J.A., M. Richardson, S. Telizyn, and J. Bienenstock. 1983. Basophil/mast cell precursors in human peripheral blood. Blood 61: 775-780.

Dennert, G., R. Hyman, J. Lesley, and I.S. Trowbridge. 1980. Effects of cytotoxic monoclonal antibody specific for T200 glycoprotein on functional lymphoid cell populations. Cell. Immunol. 53: 350-364.

Desaga, J.F., M.R. Parwaresch, and H.K. Muller-Hermelink. 1971. Die zytochemische identifikation der mastzellvorstufen bei der ratte. Z. Zellforsch 121: 292-300.

Dey, S.K. 1981. Role of histamine in implantation: Inhibition of histidine decarboxylase induces delayed implantation in the rabbit. Biol. Reprod. 24: 867-869.

Dey, S.K., and D.C. Johnson. 1980a. Histamine formation by mouse preimplantation embryos. J. Reprod. Fert. 60: 457-460.

Dey, S.K., and D.C. Johnson. 1980b. Reevaluation of histamine in implantation. In F. Kimball (ed), The Endometrium. Spectrum Publications, New York.

Dey, S.K., D.C. Johnson, and J.G. Santos. 1979. Is histamine production in the blastocyst required for implantation in the rabbit? Biol. Reprod. 21: 1169-1174.

Djeu, J.Y., E. Lanza, S. Pastore, and A.J. Hapel. 1983. Selective growth of natural cytotoxic but not natural killer effector cells in interleukin 3. Nature 306: 788-791.

Doolittle, R.F., M. W. Hunkapiller, L.E. Hood, S.G. Devare, K.C. Robbins, S.A. Aaronson, and H.M. Antonaides. 1983. Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet derived factor. Science 221: 275-277.

Downward, J., Y. Yarden, E. Mayes, S. Scrase, N. Toddy, P. Stockwell, A. Ullrich, J. Schlessinger, and M.D. Waterfield. 1984. Close similarity of epidermal growth factor receptor and v-erb B oncogene protein sequences. Nature 307: 521-527.

Dunn, T.B., and M. Potter. 1957. A transplantable mast cell neoplasm in the mouse. J. Natl. Canc. Inst. 30: 587-601.

Dvorak, A.M., G. Nabel, K. Pyne, H. Cantor, H.F. Dvorak, and S.J. Galli. 1982. Ultrastructure of the mouse basophil. Blood 59: 1279-1285.

Efrati, P., A. Klagman, and H. Spitz. 1957. Mast cell leukemia? Malignant mastocytosis with leukemia-like manifestations. Blood 12: 869-882.

Ehrlich, P. 1877. Bietrage zur Kenntnis der Anilinfarbungen und ihrer Verwendung in der Mikroskopishen Technik. Arch. f. Mikros. Anat. 13: 263-269.

Ehrlich, P. 1879. Bietrage zur Kenntis der granulierten Bindegewebszellen und der eosinophilen Leukocythen. Arch. Anat. Physiol. 3: 166-169.

Enerback, L. 1966a. Mast cells in rat gastrointestinal mucosa. 1. Effects of fixation. Acta Pathol. Microbiol. Scand. 66: 289-302.

Enerback, L. 1966b. Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. Acta Pathol. Microbiol. Scand. 66: 303-312.

Enerback, L. 1966c. Mast cells in rat gastrointestinal mucosa. 3. Reactivity towards compound 48/80. Acta Pathol. Microbiol. Scand. 66: 313-322.

Enerback, L. 1966d. Mast cells in rat gastrointestinal mucosa. 4. Monoamine storing capacity. Acta Pathol. Microbiol. Scand. 67: 365-379.

Enerback, L. 1981. The gut mucosal mast cell. Monogr. Allergy 17: 222-232.

Erjavek, F., F. Lernbeck, T. Florjanc-Irman, G. Skofitsch, J. Donnerer, A. Saria, and P. Holzer. 1981. Release of histamine by substance P. Naunym-Schmiedeberg's Arch. Pharmacol. 317: 67-70.

Farram, E., and D.S. Nelson. 1980. Mouse mast cells as anti-tumor effector cells. Cell. Immunol. 55: 294-301.

Farrar, W.L., T.P. Thomas, and W.B. Anderson. 1985. Altered cytosol/membrane enzyme redistribution on interleukin-3 activation of protein kinase C. Nature 315: 235-237.

Fauser, A.A., and H.A. Messner. 1979. Identification of megakaryocytes, macrophages, and eosinophils in colonies of human bone marrow containing neutrophilic granulocytes and erythroblasts. Blood 53: 1023-1027.

Fjellner, B., and O. Hagermark. 1981. Studies on pruritogenic and histamine-releasing effects of some putative peptide neurotransmitters. Acta Derm. Venereol. (Stockh) 61: 245-250.

Fung, M.C., A.J. Hapel, S. Ymer, D.R. Cohen, R.M. Johnson, H.D. Campbell, and I.G. Young. 1984. Molecular cloning of cDNA for murine Interleukin-3. Nature 307: 233-237.

Galli, S.J., A.M. Dvorak, T. Ishizaka, G. Nabel, H. Der Simonian, H. Cantor, and H.F. Dvorak. 1982a. A cloned cell with NK function resembles basophils by ultrastructure and expresses IgE receptors. Nature 298: 288-290.

Galli, S.J., A.M. Dvorak, J.A. Marcum, T. Ishizaka, G. Nabel, H. Der Simonian, K. Pyne, J.M. Goldin, R.D. Rosenberg, H. Cantor, and H.F. Dvorak. 1982b. Mast cell clones: A model for the analysis of cellular maturation. J. Cell. Biol. 95: 435-444.

Galli, S.J., and H.F. Dvorak. 1979. Basophils and mast cells: Structure, function, and role in hypersensitivity. In S. Gupta and R.A. Good (eds), Cellular, Molecular and Clinical Aspects of Allergic Disorders. Plenum, New York.

Galli, S.J., A.S. Galli, A.M. Dvorak, and H.F. Dvorak. 1976. Metabolic studies of guinea pig basophilic leukocytes in short-term tissue culture. I. Measurement of histamine-synthesizing capacity by using an isotopic-thin layer chromatographic assay. J. Immunol. 117: 1085-1092.

Garland, J.M., and S. Crompton. 1983. A preliminary report: Preparations containing interleukin-3 (IL-3) promote proliferation of multipotential stem cells (CFU-S) in the mouse. Exp. Hematol. 11: 757-761.

Ghiara, P., D. Boraschi, L. Villa, G. Scapigliati, C. Taddei, and A Tagliabue. 1985. In vitro generated mast cells express natural cytotoxicity against tumors. Immunology 55: 317-324.

Ginsburg, H. 1963. The in vitro differentiation and culture of normal mast cells from the mouse thymus. Ann. N.Y. Acad. Sci. 103: 20-39.

Ginsburg, H., D. Ben-Shahar, and E. Ben-David. 1982. Mast cell growth on fibroblast monolayers: Two-cell entities. Immunology 45: 371-380.

Ginsburg. H., and D. Lagunoff. 1967. The in vitro differentiation of mast cells. Culture of cells from immunized mouse lymph nodes and thoracic duct lymph on fibroblast monolayers. J. Cell. Biol. 35: 685-697.

Ginsburg, H., I. Nir, I. Hammel, R. Eren, B.-A. Weissman, and Y. Naot. 1978. Differentiation and activity of mast cells following immunization in cultures of lymph-node cells. Immunology 35: 485-502.

Ginsburg, H., E.C. Olson, T.F. Huff, H. Okudaira, and T. Ishizaka. 1981. Enhancement of mast cell differentiation in vitro by T cell factor(s). Int. Archs. Allergy Appl. Immunol. 66: 447-458.

Ginsburg, H., and L. Sachs. 1963. Formation of pure suspensions of mast cells in tissue culture by differentiation of lymphoid cells from the mouse thymus. J. Natl. Cancer Inst. 31: 1-39.

Goff, S.P., E. Gilboa, O.N. Witte, and D. Baltimore. 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: Studies with cloned viral DNA. Cell 22: 777-785.

Goldwasser, E., J.N. Ihle, M.B. Prystowsky, I. Rich, and G. VanZant. 1983. The effect of interleukin-3 on hematopoietic precursor cells. In: D.W. Golde and P.A. Marks (eds.), Normal and Neoplastic Hematopoiesis. Alan R. Liss, New York, New York.

Gonzales-Molina, A. and H.L. Spiegelberg. 1978. A subpopulation of normal human peripheral B lymphocytes that binds IgE. J. Clin. Invest. 59: 616-624.

Greenberger, J.S., P.B. Davisson, P.J. Gans, and W.C. Moloney. 1979. In vitro induction of continuous acute promyelocytic cell lines by Friend or Abelson murine leukemia virus. Blood 53: 987-1001.

Guy-Grand, D., M. Dy, G. Luffau, and P. Vassalli. 1984. Gut mucosal mast cells. Origin, traffic, and differentiation. J. Exp. Med. 160: 12-28.

Habu, S., M. Kasai, Y. Nagai, N. Tamaoki, T. Tada, L.A. Herzenberg, and K. Okumura. 1980. The glycolipid asialo GM1 as a new differentiation antigen of fetal thymocytes. J. Immunol. 125: 2284-2288.

Haig, D.M., T.A. McKee, E.E. Jarrett, R. Woodbury, and H.R. Miller. 1982. Generation of mucosal mast cells is stimulated in vitro by factors derived from T cells of helminth-infected rats. Nature 300: 188-190.

Haig, D.M., C. McMenamin, C. Gunneberg, R. Woodbury, and E.E.E. Jarrett. 1983. Stimulation of mucosal mast cell growth in normal and nude rat bone marrow cultures. Proc. Natl. Acad. Sci. USA 80: 4499-4503.

Hall, B.T. 1981. Mechanism of altered cell-mediated immune responsiveness in mice infected with Trichinella spiralis. Ph. D. dissertation, University of Florida.

Hara, H., and M. Ogawa. 1978. Murine hemopoietic colonies in culture containing normoblasts, macrophages, and megakaryocytes. A. J. Hematol. 4: 23-34.

Hasthorpe, S. 1980. A hemopoietic cell line dependent on a factor in pokeweed mitogen-stimulated spleen cell conditioned medium. J. Cell. Physiol. 105: 379-384.

Hatanaka, K., Y. Kitamura, and Y. Nishimune. 1979. Local development of mast cells from bone marrow-derived precursors in the skin of mice. Blood 53: 142-147.

Henderson, W.R., E.Y. Chi, E.C. Jong, and S.J. Klebanoff. 1981. Mast cell-mediated tumor cell cytotoxicity. Role of the peroxidase system. J. Exp. Med. 153: 520-533.

Horton, M.A., and H.A.W. O'Brien. 1983. Characterization of human mast cells in long term culture. Blood 62: 1251-1260.

Ihle, J.N.. 1985. Biochemical and biological properties of Interleukin-3: A lymphokine mediating the differentiation of a lineage of cells that includes prothymocytes and mastlike cells. Contemp. Top. Mol. Immunol. 10: 93-119.

Ihle, J.N., J. Keller, L. Henderson, F. Klein, and E. Palasynski. 1982. Procedures for the purification of interleukin 3 to homogeneity. J. Immunol. 129: 2431-2435.

Ihle, J.N., J. Keller, S. Oroszlan, L.E. Henderson, T.D. Copeland, F. Fitch, M.B. Prystowsky, E. Goldwasser, J.W. Schrader, E. Palaszynski, M. Dy, and B. Lebel. 1983. Biologic properties of homogeneous interleukin 3. I. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, P cell stimulating factor activity, colony-stimulating factor activity, and histamine-producing cell-stimulating factor activity. J. Immunol. 131: 282-287.

Ihle, J.N., L. Pepersack, and L. Rebar. 1981. Regulation of T cell differentiation. In vitro induction of 20 alpha-hydroxysteroid dehydrogenase in splenic lymphocytes from athymic mice by a unique lymphokine. J. Immunol. 126: 2184-2189.

Iscove, N.N. 1985. Specificity of hemopoietic growth factors. In I.L. Weissman (ed.), Leukemia (Dahhlem Workshop Reports, Vol. 30). Springer-Verlag, Berlin.

Iscove, N.N., and C. Roitsch. 1985. The multilineage hemopoietic growth factor (MultiHGF/BPA/MCGF/IL3). In C. Song and A. Schimpl (eds.). Cellular and Molecular Biology of Lymphokines. Academic Press, Orlando, FL.

Ishizaka, T., T. Adaci, T. Chang, and K. Ishizaka. 1977. Development of mast cells in vitro. II. Biologic function of cultured mast cells. J. Immunol. 118: 211-217.

Ishizaka, T., H. Okudaira, L.E. Mauser, and K. Ishizaka. 1976. Development of rat mast cells in vitro. I. Differentiation of mast cells from thymus cells. J. Immunol. 116: 747-754.

Jarrett, E.E.E., and D.M. Haig. 1984. Mucosal mast cells in vivo and in vitro. Immunol. Today 5: 115-119.

Johnson, G.R., and D. Metcalf. 1977. Pure and mixed erythroid colony formation in vitro stimulated by spleen conditioned medium with no detectable erythropoietin. Proc. Natl. Acad. Sci. USA 74: 3879.

Kahlson, G., and E. Rosengren. 1968. New approaches to the physiology of histamine. Physiol. Rev. 48: 155-196.

Kasai, M., M. Iwamori, Y. Nagai, K. Okumura, and T. Tada. 1980. A glycolipid on the surface of mouse natural killer cells. Eur. J. Immunol. 10: 175-180.

Kasai, M., T. Takashi, T. Takahashi, and T. Tokunaga. 1983. A new differentiation antigen (FT-1) shared with fetal thymocytes and leukemia cells in the mouse. J. Exp. Med. 159: 971-980.

Katz, H.R., P.A. LeBlanc, and S. Russell. 1983. Two classes of mast cells delineated by monoclonal antibodies. Proc. Natl. Acad. Sci. USA 80: 5916-5918.

Katz, H.R., R.L.. Stevens, and K.F. Austen. 1985a. Heterogeneity of mammalian mast cells differentiated in vivo and in vitro. J. Allergy Clin. Immunol. 76: 250-259.

Katz, H.R., G.A. Schwarting, P.A. LeBlanc, K.F. Austen, and R.L. Stevens. 1985b. Identification of the neutral glycosphingolipids of murine mast cells: Expression of Forrsman glycolipid by the serosal but not the bone marrow-derived subclass. J. Immunol. 134: 2617-2623.

Kay, H.D., G.D. Bonnard, W.H. West, and R.B. Herberman. 1977. A functional comparison of human Fc-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. J. Immunol. 118: 2058-2066.

Kennedy, T.G. 1977. Evidence for a role for prostaglandin in the initiation of blastocyst implantation in the rat. Biol Reprod. 16: 286-291.

Kiernan, J.A. 1972. The involvement of mast cells in vasodilatation due to axon reflexes in injured skin. Q. J. Exp. Physiol. 57: 311-317.

Kincade, P.W., G. Lee, C.J. Paige, and M.P. Scheid. 1981a. Cellular interactions affecting the maturation of murine B-lymphocyte precursors in vitro. J. Immunol. 127: 255-260.

Kincade, P.W., G. Lee, T. Watanabe, L. Sun, and M.P. Scheid. 1981b. Antigens displayed on murine B lymphocyte precursors. J. Immunol. 127: 2262-2268.

King, S.J., H.R.P. Miller, G.F.J. Newlands, and R.G. Woodbury. 1985. Depletion of mast cell protease by corticosteroids: Effect on intestinal anaphylaxis in the rat. Proc. Natl. Acad. Sci. USA 82: 1214-1218.

King-Smith, E.A., and A. Morley. 1970. Computer simulation of granulopoiesis. Normal and impaired granulopoiesis. Blood 36: 254-262.

Kitamura, Y., S. Go, and K. Hatanaka. 1978. Decrease of mast cells in W/W^V mice and their increase by bone marrow transplantation. Blood 52: 447-452.

Kitamura, Y., K. Hatanaka, M. Murakami, and H. Shibata. 1979a. Presence of mast cell precursors in peripheral blood of mice demonstrated by parabiosis. Blood 53: 1085-1088.

Kitamura, Y., M. Shimada, and S. Go. 1979c. Presence of mast cell precursors in fetal liver of mice. Dev. Biol. 70: 510-514.

Kitamura, Y., M. Shimada, S. Go, H. Matsuda, K. Hatanaka, and M. Seki. 1979d. Distribution of mast cell precursors in hematopoietic and lymphopoietic tissues of mice. J. Exp. Med. 150: 482-490.

Kitamura, Y., M. Shimada, K. Hatanaka, and Y. Miyano. 1977. Development of mast cells from grafted bone marrow cells in irradiated mice. Nature 268: 442-443.

Kitamura, Y., M. Yokoyama, H. Matsuda, and T. Ohno. 1981. Spleen colony-forming cell as common precursor for tissue mast cells and granulocytes. Nature 291: 159-160.

Kruger, G., W. Sterry, and B.M. Czarnetzki. 1983. Cultures of mast cell-like (MCL) cells from human pleural exudate cells. Blut 46: 143-148.

Lagunoff, D. 1972. Contributions of electron microscopy to the study of mast cells. J. Invest. Dermatol. 58: 296-311.

Lagunoff, D. 1985. The mast cell. In E.B. Weiss, M.S. Segal, and M. Stein (eds.), Bronchial Asthma: Mechanisms and Therapeutics. Little, Brown and Company, Boston, MA.

Lagunoff, D., and Y.C. Chi. 1980. Cell biology of mast cells and basophils. In G. Weissman (ed.), Cell Biology of Inflammation. Handbook of Inflammation, Vol.2. Elsevier/North-Holland, Amsterdam.

Lala, P.K., S. Chatterjee-Hasrouni, M. Kearns, B. Montgomery, and V. Colavincenzo. 1983. Immunobiology of the fetal-maternal interface. Immunol. Rev. 75: 87-116.

Lattime, E.C., G.A. Pecoraro, and O. Stutman. 1983. The activity of natural cytotoxic cells is augmented by interleukin 2 and interleukin 3. J. Exp. Med. 157: 1070-1075.

LeBlanc, P.A., S.W. Russell, and S.-M.T. Chang. 1982. Mouse mononuclear phagocyte antigenic heterogeneity detected by monoclonal antibodies. J. Reticuloendothel. Soc. 32: 219-231.

Lewis, R.A., and K.F. Austen. 1981. Mechanism of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. Nature 293: 103-108.

Lewis, R.G., A.F. Spencer, and J.E. Silbert. 1973. Biosynthesis of glycosaminoglycans by cultured mastocytoma cells. Biochem. J. 134: 455-463.

Lorenz, W., A. Schauer, S. Heitland, R. Calvoer, and E. Werle. 1969. Biochemical and histochemical studies on the distribution of histamine in the digestive tract of man, dog, and other mammals. Arch. Pharmakol. 265: 81-100.

Maniatis, T., E. Fritsch, and J. Sambrook (eds). 1982. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, New York.

Marx, J.L. 1984. A new view of receptor action. Science 224: 271-274.

Maximow, A. 1906. Uber die zellformen des lockeren Bindegewebes. Arch. f. Mikr. Anat. 67: 680-757.

Mayrhofer, G. 1979a. The nature of the thymus dependency of mucosal mast cells. I. An adaptive secondary response to challenge with Nippostrongylus brasiliensis. Cell. Immunol. 47: 304-311.

Mayrhofer, G. 1979b. The nature of the thymus dependency of mucosal mast cells. II. The effect of thymectomy and of depleting recirculating lymphocytes on the response to Nippostrongylus brasiliensis. Cell. Immunol. 47: 312-322.

Mayrhofer, G., and H. Bazin. 1981. Nature of the thymus dependency of mucosal mast cells. III. Mucosal mast cells in nude mice and nude rats, in B rats and in a child with Di George syndrome. Int. Archs. Allergy Appl. Immunol. 64: 320-331.

Mayrhofer, G., and R. Fisher. 1979. Mast cells in severely T-cell depleted rats and the response to infestation with Nippostrongylus brasiliensis. Immunology 37: 145-155.

McCarthy, J.H., T.E. Mandel, O.M. Garson, and D. Metcalf. 1980. The presence of mast cells in agar cultures. Exp. Hematol. 8: 562-567.

McMenamin, C., E.E.E. Jarrett, and A. Sanderson. 1985. Surface phenotype of T cells producing growth of mucosal mast cells in normal rat bone marrow culture. Immunology 55: 399-403.

Melander, A. 1977. Aminergic regulation of thyroid activity: Importance of the sympathetic innervation and of the mast cells of the thyroid gland. Acta Med. Scand. 201: 257-262.

Melchers, F. 1979. Murine embryonic B lymphocyte development in the placenta. Nature 277: 219-221.

Melchers, F., and J. Abramczuk. 1980. Murine embryonic blood between 10 and 13 of gestation as a source of immature B cells. Eur. J. Immunol. 10: 763-767.

Melewicz, F.M., J.M. Plummer, and H.L. Spiegelberg. 1982. Comparison of the Fc receptors for IgE on human lymphocytes and monocytes. J. Immunol. 129: 563-569.

Mendoza, G.R., and H. Metzger. 1976. Disparity of IgE binding between normal and tumor mouse mast cells. J. Immunol. 117: 1573-1578.

Metcalf, D., and A. Kelso. 1985. Production of colony stimulating factors by murine T lymphocytes. In C. Sorg and A. Schimpl (eds). Cellular and Molecular Biology of Lymphokines. Academic Press, Inc., Orlando, FL.

Metcalf, D., and M.A.S. Moore. 1971. Embryonic aspects of haemopoiesis. In D. Metcalf and M.A.S. Moore (eds.), Haemopoietic Cells. North-Holland Publishing Company, Amsterdam.

Metcalfe, D.D., M. Kaliner, and M.A. Donlon. 1981. The mast cell. CRC Crit. Rev. Immunol. 3: 23-74.

Metcalfe, D.D., R.A. Lewis, J.E. Silbert, R.D. Rosenberg, S.I. Wasserman, and K.F. Austen. 1979. J. Clin. Invest. 64: 1537-1543.

Metcalfe, D.D., S.I. Wasserman, and K.F. Austen. 1980b. Isolation and characterization of sulfated mucopolysaccharides from rat leukaemic (RBL-1) basophils. Biochem. J. 185: 367-372.

Michell, B. 1984. Oncogenic intelligence: Oncogenes and inositol lipids. Nature 308: 770.

Michels, W.A. 1963. The mast cells. Ann. N.Y. Acad. Sci. 103: 235-372.

Mishell, B.B., and S.M. Shiigi (eds.). 1980. Preparation and testing of reagents. In Selected Methods in Cellular Immunology. W.H. Freeman and Co., San Francisco, CA.

Moore, M.A.S., and D. Metcalf. 1970. Ontogeny of the haematopoietic system: Yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. Brit. J. Haematol. 18: 279-296.

Mori, Y., H. Akedo, K. Tanaka, Y. Tanigaki, and M. Okada. 1979. Effect of sodium butyrate on the granulopoiesis of mastocytoma cells. Exp. Cell Res. 118: 15-22.

Morse, H.C. III, W.F. Davidson, R.A. Yetter, E.D. Murphy, J.B. Roths, and R.L. Coffman. 1982. Abnormalities induced by the mutant gene lpr: Expansion of a unique lymphocyte subset. Proc. Natl. Acad. Sci. USA 129: 2612-2615.

Muller, R., D.J. Slamon, J.M. Tremblay, M.J. Cline, and I.M. Verma. 1982. Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. Nature 299: 640-644.

Muramatsu, T., H. Muramatsu, M. Kasai, S. Habu, and K. Okumura. 1980. Receptors for Dolichus bifloris agglutinin: New cell surface markers on a spontaneous leukemia. Biochem. Biophys. Res. Commun. 96: 1547-1553.

Nabel, G., S.J. Galli, A.M. Dvorak, H.F. Dvorak, and H. Cantor. 1981. Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. Nature 291: 332-334.

Nagao, K., K. Yokoro, and S.A. Aaronson. 1981. Continuous lines of basophil/mast cells derived from normal mouse bone marrow. Science 212: 333-335.

Nakahata, T., and M. Ogawa. 1982. Identification in culture of a class of hemopoietic colony forming units with extensive capability to self-renew and generate multipotential hemopoietic colonies. Proc. Natl. Acad. Sci. USA 79: 3843-3847.

Nakahata, T., A.J. Gross, and M. Ogawa. 1982a. A stochastic model of self-renewal and commitment to differentiation of the primitive hemopoietic stem cells in culture. J. Cell. Physiol. 113: 455-458.

Nakahata, T., S.S. Spicer, J.R. Cantey, and M. Ogawa. 1982b. Clonal assay of mouse mast cell colonies in methylcellulose culture. Blood 60: 352-361.

Nakano, T., T. Sonoda, C. Hayashi, A. Yamatodani, Y. Kanayama, T. Yamamura, A. Asai, T. Yonezawa, Y. Kitamura, and S.J. Galli. 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/W^V mice. Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. J. Exp. Med. 162: 1025-1043.

Nalbandov, A.V. 1971. Endocrine control of implantation. In R.J. Blandau (ed), Biology of the Blastocyst. University of Chicago Press, Chicago, IL.

Nawa, Y., and H.R.P. Miller. 1979. Adoptive transfer of the intestinal mast cell response in rats infected with Nippostrongylus brasiliensis. Cell. Immunol. 42: 225-239.

Newson, B., A. Dahlstrom, L. Enerback, and H. Ahlman. 1983. Suggestive evidence for a direct innervation of mucosal mast cells. Neuroscience 10: 565-570.

Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 308: 693-698.

O'Farrell, P.H., E. Kutter, and M. Nakanishi. 1980. A restriction map of the bacteriophage T4 genome. Molec. Gen. Genet. 179: 421-435.

Ogawa, M., T. Nakahata, A.G. Leary, A.R. Sterk, K. Ishizaka, and T. Ishizaka. 1983. Suspension culture of human mast cells/basophils from umbilical cord blood mononuclear cells. Proc. Natl. Acad. Sci. USA 80: 4494-4498.

Olson, E.C., and D.A. Levy. 1976. Thymus-dependency of the mast cell response to Nippostrongylus brasiliensis in mice. Fed. Proc. 35: 491.

Olsson, I., B. Berg, L.A. Fransson, and A. Norden. 1970. The identity of the metachromatic substance of basophilic leucocytes. Scand. J. Haematol. 7: 440-444.

Omary, M.B., I.S. Trowbridge, and M.P. Scheid. 1980. T200 cell surface glycoprotein of the mouse. Polymorphism defined by the Ly-5 system of alloantigens. J. Exp. Med. 151: 1311-1316.

Orenstein, N.S., S.J. Galli, A.M. Dvorak, J.E. Silbert, and H.F. Dvorak. 1978. Sulfated glycosaminoglycans of guinea pig basophilic leukocytes. J. Immunol. 121: 586-592.

Padawer, J. 1974. Mast cells: Extended life span and lack of granule turnover under normal in vivo conditions. Exp. Mol. Pathol. 20: 269-280.

Palacios, R., G. Henson, M. Steinmetz, and J.P. McKearn. 1984. Interleukin-3 supports the growth of mouse pre-B clones in vitro. Nature 309: 126-131.

Parmley, R.T., M. Ogawa, S.S. Spicer, and N.J. Wright. 1976. Ultrastructure and cytochemistry of bone marrow granulocytes in culture. Exp. Hematol. 4: 75-89.

Pearce, F.L. 1982. Functional heterogeneity of mast cells from different species and tissues. Klin. Wschr. 60: 954-957.

Pearce, F.L. 1983. Mast cell heterogeneity. Trends Pharmacol. Sci. 4: 165-167.

Pearce, F.L., H. Ali, K.E. Barrett, A.D. Befus, J. Bienenstock, J. Brostoff, M. Ennis, K.C. Flint, B. Hudspith, N.M. Johnson, K.B.P. Leung, and P.T. Peachell. 1985. Functional characteristics of mucosal and connective tissue mast cells of man, the rat and other animals. Int. Archs. Allergy Appl. Immunol. 77: 274-276.

Pearce, F.L., A.D. Befus, J. Gauldie, and J. Bienenstock. 1982. Effects of anti-allergic compounds on histamine secretion by isolated intestinal mast cells. J. Immunol. 128: 2481-2486.

Pharr, P.N., T. Suda, K.L.Bergmann, L.A. Avila, and M. Ogawa. 1984. Analysis of pure and mixed mast cell cultures. J. Cell. Physiol. 120: 1-12.

Pierce, J.H., P.P. Di Fiore, S.A. Aaronson, M. Potter, J. Pumphrey, A. Scott, and J.N. Ihle. 1985. Neoplastic transformation of mast cells by Abelson-MuLV: Abrogation of IL-3 dependence by a nonautocrine mechanism. Cell 41: 685-693.

Plaut, M., L.M. Lichtenstein, E. Gillespie, and C.S. Henney. 1973. Studies on the mechanism of lymphocyte-mediated cytolysis. IV. Specificity of the histamine receptor on effector T cells. J. Immunol. 111: 389-394.

Pluznik, D.H., and L. Sachs. 1965. The cloning of normal "mast" cells in tissue culture. Cell. and Comp. Physiol. 66: 319-324.

Potter, M., M.D. Sklar, and W.P. Rowe. 1973. Rapid viral induction of plasmacytomas in pristane-primed BALB/c mice. Science 182: 592-594.

Premkumar, E., M. Potter, P.A. Singer, and M.D. Sklar. 1975. Synthesis, surface deposition, and secretion of immunoglobulins by Abelson virus-transformed lymphosarcoma cell lines. Cell 6: 149-159.

Pretlow, T.G., and I.M. Cassidy. 1970. Separation of mast cells in successive stages of differentiation using programmed gradient sedimentation. Am J. Pathol. 61: 323-339.

Raff, M.C., J.J.T. Owen, M.D. Cooper, A.R. Lawton, M. Megson, and W.E. Gathings. 1975. Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin-induced immunoglobulin suppression in vitro. Possible implications for B-cell tolerance to self. J. Exp. Med. 142: 1052-1064.

Raghupathy, R., B. Singh, and T.G. Wegmann. 1984. Fate of antipaternal H-2 antibodies bound to the placenta in vivo. Transplantation 37: 296-300.

Ralph, P., M.A.S. Moore, and K. Nilsson. 1976. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. Exp. Med. 143: 1528-1533.

Rapp, U.R., J.L. Cleveland, K. Brightman, A. Scott, and J.N. Ihle. 1985. Abrogation of IL-3 and IL-2 dependence by recombinant murine retroviruses expressing v-myc oncogenes. Nature 317: 434-438.

Raschke, W.C., S. Baird, P. Ralph, and I. Nakoinz. 1978. Functional macrophage cell lines transformed by Abelson leukemia virus. Cell 15: 261-267.

Razin, E., C. Cordon-Cardo, and R. A. Good. 1981a. Growth of a pure population of mouse mast cells in vitro with conditioned medium derived from concanavalin A-stimulated splenocytes. Proc. Natl. Acad Sci. USA 78: 2559-2561.

Razin, E., C. Cordon-Cardo, C.R. Minick, and R.A. Good. 1982a. Studies of the exocytosis of cultured mast cells derived from mouse bone marrow. Exp. Hematol. 10: 524-532.

Razin, E., J.N. Ihle, D. Seldin, J.-M. Mencia-Huerta, H.R. Katz, P.A. LeBlanc, A. Hein, J.P. Caulfield, K. F. Austen, and R. L. Stevens. 1984a. Interleukin 3: A differentiation and growth factor for the mouse mast cell that contains chondroitin sulfate E proteoglycan. J. Immunol. 132: 1479-1486.

Razin, E., J.M. Mencia-Huerta, R.A. Lewis, E.J. Corey, and K.F. Austen. 1982b. Generation of leukotriene C4 from a subclass of mast cells differentiated in vitro from mouse bone marrow. Proc. Natl. Acad. Sci. USA 79: 4665-4667.

Razin, E., J.-M. Mencia-Huerta, R.L. Stevens, R.A. Lewis, F.-T. Liu, E.J. Corey, and K.F. Austen. 1983. IgE-mediated release of leukotriene C4, chondroitin sulfate E proteoglycan, beta-hexosaminidase, and histamine from cultured bone marrow-derived mouse mast cells. J. Exp. Med. 157: 189-201.

Razin, E., A.B.Rifkind, C. Cordon-Cardo, and R.A. Good. 1981b. Selective growth of a population of human basophils in vitro. Proc. Natl. Acad. Sci. USA 78: 5793-5796.

Razin, E., R.L. Stevens, F. Akiyama, K. Schmid, and K.F. Austen. 1982c. Culture from mouse bone marrow of a subclass of mast cells possessing a distinct chondroitin sulfate proteoglycan with glycosaminoglycans rich in N-acetylgalactosamine-4,6-disulfate. J. Biol. Chem. 257: 7229-7236.

Razin, E. R.L. Stevens, K.F. Austen, J.P. Caulfield, A. Hein, F.-T. Liu, M. Clabby, G. Nabel, H. Cantor, and S. Friedman. 1984b. Cloned mast cells derived from immunized lymph node cells and from foetal liver cells exhibit characteristics of bone marrow-derived mast cells containing chondroitin sulfate E proteoglycan. Immunology 52: 563-575.

Recklinghausen, F. von. 1863. Ueber Eiter- und Bindegewebskorperchen. Virchow Arch. Pathol. Anat. 28: 157-164.

Reed, N.D., P.K. Crowle, R.S. Booth, and J.J. Munoz. 1982. Thymus dependence of specific IgE production and intestinal mast cell accumulation. In N.D. Reed (ed), Proceedings of the Third International Workshop on Nude Mice. Fisher, New York.

Rees, W.D.W., J. Rhodes, M.H. Wheeler, J.V. Pfaila, A.C. Esterhuizen, and R.G. Newcombe. 1981. Effect of compound 48/80 on canine gastric mucosa. Digestion 22: 39-46.

Remacle-Bonnet, M.M., R.J. Rance, and R.C. Depieds. 1983. Non-specific immunoregulatory factors in the cytosol of human trophoblast. J. Reprod. Immunol. 5: 123-134.

Rennick, D.M., F.D. Lee, T. Yokota, K.I. Arai, H. Cantor, and G. Nabel. 1985. A cloned mast cell growth factor cDNA encodes a multilineage hematopoietic growth factor: Multiple activities of interleukin 3. J. Immunol. 134: 910-914.

Rigby, P.W.J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. 113: 237-251.

Risser, R., M. Potter, and W.P. Rowe. 1978. Abelson virus-induced lymphomagenisis in mice. J. Exp. Med. 148: 714-726.

Robbins, K.C., H. Antoniades, S.G. Devare, M.W. Hunkapiller, and S.A. Aaronson. 1983. Structural and immunological similarities between simian sarcoma virus gene product(s) and human platelet-derived growth factor. Nature 305: 605-608.

Robin, J.-L., D. Seldin, K.F. Austen, and R.A. Lewis. 1985. Regulation of mediator release from mouse bone marrow-derived mast cells by glucocorticoids. J. Immunol. 135: 2719-2726.

Rocklin, R.E. 1976. Modulation of cellular immune responses in vivo and in vitro by histamine receptor-bearing lymphocytes. J. Clin. Invest. 57: 1051-1058.

Roe, R., and S.C. Bell. 1982. Humoral immune responses in murine pregnancy. II. Kinetics and nature of the response in females pre-immunized against paternal antigens. Immunology 46: 23-30.

Rose, M.L., D.V.M. Parrott, and R.G. Bruce. 1976. Migration of lymphoblasts to the small intestine. I. Effect of Trichinella spiralis infection on the migration of mesenteric lymphoblasts and mesenteric T lymphoblasts in syngeneic mice. Immunology 31: 723-730.

Rosenberg, N., and D. Baltimore. 1976a. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. J. Exp. Med. 145: 1453-1463.

Rosenberg, N., and D. Baltimore. 1976b. In vitro lymphoid cell transformation by Abelson murine leukemia virus. In D. Baltimore, A.S. Huang, and C.F. Fox (eds). Animal Virology. Academic Press, New York, NY.

Rosenberg, N., and D. Baltimore. 1980. Abelson virus. In G. Klein (ed.). Viral Oncology. Raven Press, New York, NY.

Rosenberg, N., D. Baltimore, and C.D. Scher. 1975. In vitro transformation of lymphoid cells by Abelson murine leukemia virus. Proc. Natl. Acad. Sci. USA 72: 1932-1936.

Ruitenberg, E.J., and A. Elgersma. 1976. Absence of intestinal mast cell response in congenitally athymic mice during Trichinella spiralis infection. Nature 264: 258-260.

Sathiamoorthy, S.S., A.K. Ganguly, and O.P. Bhatnagar. 1976. Effect of bilateral adrenalectomy and parenteral betamethasone on gastric mucosal mast cell population in albino rats. Experientia 32: 1300-1301.

Schalm, O.W., N.C. Jain, and E.J. Carroll. 1975. Materials and methods for the study of the blood, including brief comments on factors to be considered in interpretation. In Veterinary Hematology. Lea and Febiger, Philadelphia, PA.

Scheid, M.P., and D. Triglia. 1979. Further description of the Ly-5 system. Immunogenetics 9: 423-433.

Scher, C.D. and R. Siegler. 1975. Direct transformation of 3T3 cells by Abelson murine leukemia virus. Nature 253: 729-731.

Schindler, R., M. Day, and G.A. Fisher. 1959. Culture of neoplastic mast cells and their synthesis of 5-hydroxytryptamine and histamine synthesis in vitro. Cancer Res. 19: 47-51.

Schrader, J.W. 1981. The in vitro production and cloning of the P cell, a bone marrow-derived null cell that expresses H-2 and Ia antigens, has mast cell-like granules, and is regulated by a factor released by activated T cells. J. Immunol. 126: 452-458.

Schrader, J.W., F. Battye, and R. Scollay. 1982. Expression of Thy-1 antigen is not limited to T cells in cultures of mouse hemopoietic cells. Proc. Natl. Acad. Sci. USA 79: 4161-4165.

Schrader, J.W., I. Clark-Lewis, and P. Bartlett. 1980. Lymphoid stem cells. In R.P. Gale and C.F.Fox (eds.), ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 17. Academic Press, New York.

Schrader, J.W., I. Clark-Lewis, R.M. Crapper, and G.W. Wong. 1983a. P-cell stimulating factor: Characterization, action on multiple lineages of bone marrow-derived cells and role in oncogenesis. Immunol. Rev. 76: 79-104.

Schrader, J.W., I. Clark-Lewis, R.M. Crapper, G.H.W. Wong, and S. Schrader. 1985. P cell stimulating factor: biochemistry, biology, and role in oncogenisis. Contemp. Top. Mol Immunol. 10: 121-146.

Schrader, J.W., and R.M. Crapper. 1983. Autogenous production of a hemopoietic growth factor, persisting-cell-stimulating factor, as a mechanism for transformation of bone marrow-derived cells. Proc. Natl. Acad. Sci. USA 80: 6892-6896.

Schrader, J.W., S.J. Lewis, I. Clark-Lewis, and J.G. Culvenor. 1981. The persisting (P) cell: Histamine content, regulation by a T-cell derived factor, origin from a bone marrow precursor, and relationship to mast cells. Proc. Natl. Acad. Sci. USA 78: 323-327.

Schrader, J.W., and G.J.V. Nossal. 1980. Strategies for the analysis of accessory cell function: The in vitro cloning and characterization of the P cell. Immunol. Rev. 53: 61-85.

Schrader, J.W., R. Scollay, and F. Battye. 1983b. Intramucosal lymphocytes of the gut: Lyt 2 and Thy 1 phenotype of the granulated cells and evidence for the presence of both T cells and mast cell precursors. J. Immunol. 130: 558-564.

Schwartz, A., P.W. Askenase, and R.K. Gershon. 1980. Histamine inhibition of the in vitro induction of cytotoxic T-cell responses. Immunopharmacology 2: 179-190.

Sefton, B.M., T. Hunter, E.H. Ball, and S.J. Singer. 1981a. Vinculin: A cytoskeletal target of the transforming gene of Rous sarcoma virus. Cell 24: 165-174.

Sefton, B.M., T. Hunter, and W.C. Raschke. 1981b. Evidence that the Abelson virus protein functions in vivo as a protein kinase which phosphorylates tyrosine. Proc. Natl. Acad. Sci. USA 78: 1552-1556.

Selye, H. 1965. The Mast Cells. Butterworth, Washington, D.C.

Serunian, L.A., and N. Rosenberg. 1986. Abelson virus potentiates long-term growth of mature B lymphocytes. Mol. Cell. Biol. 6: 183-194.

Shanahan, F., J.A. Denburg, J. Bienenstock, and A.D. Befus. 1984. Mast cell heterogeneity. Can. J. Physiol. Pharmacol. 62: 734-737.

Shelesnyak, M.C. 1960. Nidation of the fertilized ovum. Endeavor 19: 81-86.

Shields, A., S. Goff, M. Paskind, G. Otto, and D. Baltimore. 1979. Structure of the Abelson murine leukemia virus genome. Cell 18: 955-962.

Shinefeld, L.A., V.L. Sato, and N. E. Rosenberg. 1980. Monoclonal rat anti-mouse brain antibody detects Abelson murine leukemia virus target cells in mouse bone marrow. Cell 20: 11-17.

Siden, E.J., D. Baltimore, D. Clark, and N.E. Rosenberg. 1979. Immunoglobulin synthesis by lymphoid cells transformed in vitro by Abelson murine leukemia virus. Cell 16: 389-396.

Siden, E.J. and M.L. Siegel. 1986. A simple method for the simultaneous detection of Fc receptors and differentiation markers on normal and neoplastic murine hematopoietic cells. J. Immunol. Methods, in press.

Sidman, C.L., and E.R. Unanue. Control of proliferation and differentiation in B lymphocytes by anti-Ig antibodies and a serum-derived cofactor. Proc. Natl. Acad. Sci. USA 75: 2401-2405.

Siegel, M.L., R.J. Horwitz, T.D. Morris, R.M. DiVenere, and E.J. Siden. 1985. Isolation and characterization of Abelson murine leukemia virus transformed mast cell lines from midgestation embryonic placenta. Eur. J. Immunol. 15: 1136-1141.

Silverstone, A.E., N. Rosenberg, D. Baltimore, V.L. Sato, M.P. Scheid, and E.A. Boyse. 1978. Correlating terminal deoxynucleotidyl transferase and cell-surface markers in the pathway of lymphocyte ontogeny. In B. Clarkson, P. Marks, and J. Till (eds.), Differentiation of Normal and Neoplastic Hematopoietic Cells. Cold Spring Harbor Laboratory, New York.

Sklar, M.D., E.M. Shevach, I. Green, and M. Potter. 1975. Transplantation and preliminary characterization of lymphocyte surface markers of Abelson murine leukemia virus-induced lymphomas. Nature 253: 550-552.

Soll, A.H., K.J. Lewin, and M.A. Beavin. 1981. Isolation of histamine-containing cells from rat gastric mucosa: Biochemical and morphologic differences from mast cells. Gastroenterology 80: 717-727.

Sonoda, T., Y. Kitamura, Y. Haku, H. Hara, and K.J. Mori. 1983. Mast cell precursors in various haematopoietic colonies of mice produced in vivo and in vitro. Brit. J. Haematol. 53: 611-620.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503-517.

Spivak, J.L., R.R.L. Smith, and J.N. Ihle. 1985. Interleukin 3 promotes the in vitro proliferation of murine pluripotent hematopoietic stem cells. J. Clin. Invest. 76: 1613-1621.

Sredni, B., M.M. Friedman, C.A. Bland, and D.D. Metcalfe. 1983. Ultrastructural, biochemical, and functional characteristics of histamine-containing cells cloned from mouse bone marrow: Tentative identification as mucosal mast cells. J. Immunol. 131: 915-922.

Stadler, B.M., K. Hirai, K. Tadokoro, and A.L. de Weck. 1985. Distinction of the human basophil promoting activity from human interleukin 3. Int. Archs. Allergy Appl. Immunol. 77: 151-154.

Steffen, D., S. Bird, W.P.Rowe, and R.A. Weinberg. 1979. Identification of DNA fragments carrying ecotropic proviruses of AKR mice. Proc. Natl. Acad. Sci. USA 76: 4554-4558.

Sterry, W., and B.M. Czarnetzki. 1982. In vitro differentiation of rat peritoneal macrophages into mast cells: An enzymecytochemical study. Blut 44: 211-220.

Stevens, R.L., and K.F. Austen. 1981. Proteoglycans of the mast cell. In E.L. Becker, A.S. Simon, and K.F. Austen (eds.). Biochemistry of the Acute Allergic Reaction: Fourth International Symposium (Kroc Foundation Series, Vol. 14). Alan R. Liss, New York.

Stevens, R.L., and K.F. Austen. 1982. Effect of para-nitrophenyl-beta-D-xyloside on proteoglycan and glycosaminoglycan biosynthesis in rat serosal mast cell cultures. J. Biol Chem. 257: 253-259.

Suda, T., J. Suda, and M. Ogawa. 1984. Disparate differentiation in mouse hemopoietic colonies derived from paired progenitors. Proc. Natl. Acad. Sci. USA 81: 2520-2524.

Suda, T., J. Suda, S.S. Spicer, and M. Ogawa. 1985. Proliferation and differentiation in culture of mast cell progenitors derived from mast cell deficient mice of the genotype W/W^V. J. Cell. Physiol. 122: 187-192.

Tadokoro, K., B.M. Stadler, and A.L. de Weck. 1983. Factor dependent in vitro growth of human normal bone marrow-derived basophil like cells. J. Exp. Med. 158: 857-871.

Tas, J., and R.G. Berndsen. 1977. Does heparin occur in mucosal mast cells of the rat small intestine? J. Histochem. Cytochem. 25: 1058-1062.

Taylor, K.M., S. Krilis, and B.A. Baldo. 1980. An enzymatic-isotopic microassay for measuring allergic release of histamine from blood and mast cells in vitro. Int. Archs. Allergy Appl. Immun. 61: 19.

Tertian, G., Y.-P. Yung, D. Guy-Grand, and M.A.S. Moore. 1981. Long-term in vitro culture of murine mast cells. I. Description of a growth factor-dependent culture technique. J. Immunol. 127: 788-794.

Tertian, G., Y.-P. Yung, and M.A.S. Moore. 1980. Induction and long-term maintenance of Thy-1 positive lymphocytes: Derivation from continuous marrow cultures. J. Supramol. Struct. Cell. Biol. 13: 533-539.

Thompson, S.W., and R.D. Hunt. 1966. Romanowsky-type staining technics. Wright's stain, Giemsa's stain, May-Grunwald Giemsa. In Selected Histochemical and Histopathological Methods. Second Edition. Thomas Co., Springfield, IL.

Till, J.E., and E.A. McCulloch. 1961. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Rad. Res. 12: 214-222.

Tyan, M.L. 1968. Studies on the ontogeny of the mouse immune system. I. Cell-bound immunity. J. Immunol. 100: 535-542.

Uchanska-Ziegler, B., P.Wernet, and A. Ziegler. 1982. A single-step bacterial binding assay for the classification of cell types with surface antigen-directed monoclonal antibodies. Brit. J. Haematol. 52: 155-160.

Van Den Hooff, A., and W. Tichelar-Guttar. 1983. Stromal changes in experimental carcinogenesis in the skin. I. Ultrastructural aspects of collagen degradation. Med. Pract. 86: 170-190.

Wahl, G.M., M. Stern, and G.R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazo-benzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA 76: 3683-3687.

Waneck, G.L., and N. Rosenberg. 1981. Abelson leukemia virus induces lymphoid and erythroid colonies in infected fetal cell cultures. Cell 26: 79-89.

Waterfield, M.D., G. Scrase, N. Whittle, P. Stroobant, A. Johnson, A. Wasteson, B. Westermark, C.H. Heldin, J.S. Huang, and T.F. Deuel. 1983. Platelet-derived growth factor is structurally related to the putative transforming protein $P28^{sis}$ of simian sarcoma virus. Nature 304: 35-39.

Wedling, F., M. Shreeve, D. McLeod, and A. Axelrad. 1985. A self-renewing, bipotential erythroid/mast cell progenitor in continuous cultures of normal murine bone marrow. J. Cell. Physiol. 125: 10-18.

Wedling, F., M.M. Shreeve, D. McLeod, and A. Axelrad. 1983. Long-term culture of murine erythropoietic progenitors in the absence of an adherent layer. Nature 305: 625-627.

Wegmann, T.G., B. Singh, and G.A. Carlson. 1979. Allogeneic placenta is a paternal strain immunoadsorbent. J. Immunol. 122: 270-274.

Weisner-Menzel, L., B. Schulz, F. Vakilzadeh, and B.M. Czarnetzki. 1981. Electron microscopal evidence for a direct contact between nerve fibres and mast cells. Acta Derm. Venereol. 61: 465.

Whetton, A.D., G.W. Bazill, and T.M. Dexter. 1985. Hemopoietic growth factor: Characterization and mode of action. In C. Sorg and A. Schimpl (eds.), Cellular and Molecular Biology of Lymphokines. Academic Press, Orlando, FL.

Whitlock, C.A., S.F. Zeigler, L.J. Treiman, J.I. Stafford, and O.N. Witte. 1983. Differentiation of cloned populations of immature B cells after transformation with Abelson murine leukemia virus. Cell 32: 903-911.

Wingren, U., and L. Enerback. 1983. Mucosal mast cells of the rat intestine: A re-evaluation of fixation and staining properties, with special reference to protein blocking and solubility of the granular glycosaminoglycan. Histochem. J. 15: 571-582.

Witte, O.N., A. Dasgupta, and D. Baltimore. 1980. Abelson murine leukemia virus is phosphorylated in vitro to form phosphotyrosine. Nature 283: 826-831.

Witte, O.N., N. Rosenberg, and D. Baltimore. 1979a. Identification of a normal cell protein cross-reactive to the major Abelson murine leukemia virus gene product. Nature 281: 396-398.

Witte, O.N., N. Rosenberg, and D. Baltimore. 1979b. Preparation of syngeneic tumor regressor serum reactive with the unique determinants of the Abelson MuLV encoded P120 protein at the cell surface. J. Virol. 31: 776-784.

Wlodarski, K. 1976. Mast cells in the pinna of BALB/c "nude" (nu/nu) and heterozygous (nu/+) mice. Experientia 32: 1591-1592.

Wong, G.H.W., I. Clark-Lewis, J.L. Mc Kimm-Breschkin, and J.W. Schrader. 1982. Interferon gamma-like molecule induces Ia antigens on cultured mast cell progenitors. Proc. Natl. Acad. Sci. USA 79: 6989-6993.

Woodbury, R.G., and H. Neurath. 1980. Mast cell proteases. In H. Holber (ed.), Metabolic Interconversions of Enzymes. Springer-Verlag, New York, NY.

Wysocki, L.J., and V.L. Sato. 1978. "Panning" for lymphocytes: A method for cell selection. Proc. Natl. Acad. Sci. USA 75: 2844-2848.

Yam, L.T., C.Y. Li, and W.H. Crosby. 1971. Cytochemical identification of monocytes and granulocytes. Amer. J. Clin. Path. 55: 283-290.

Yoffe, J.R., D.J. Taylor, and D.E. Woolley. 1984. Mast cell products stimulate collagenase and prostaglandin E production by cultures of adherent rheumatoid synovial cells. Biochem. Biophys. Res. Comm. 122: 270-276.

Yoffe, J.R., D.J. Taylor, and D.E. Woolley. 1985. Mast-cell products and heparin stimulate the production of mononuclear-cell factor by cultured human monocyte/macrophages. Biochem. J. 230: 83-88.

Yokota, T., F. Lee, D. Rennick, C. Hall, N. Arai, T. Mossman, G. Nabel, H. Cantor, and K. Arai. 1984. Isolation and characterization of a mouse cDNA clone that expresses mast cell growth factor activity in monkey cells. Proc. Natl. Acad. Sci. USA 81: 1070-1074.

Young, W.W., Jr., S.-I. Hakomori, J.M. Durdik, and C.S. Henney. 1980. Identification of ganglio-N-tetrasylceramide as a new cell surface marker for murine natural killer (NK) cells. J. Immunol. 124: 199-201.

Yung, Y.P., R. Eger, G.Tertian, and M.A.S. Moore. 1981. Long-term in vitro culture of murine mast cells. II. Purification of a mast cell growth factor and its dissociation from TCGF. J. Immunol. 127: 794-799.

Yung, Y.-P., and M.A.S. Moore. 1982. Long term in vitro culture of murine mast cells. III. Discrimination of mast cell growth factor and granulocyte-CSF. J. Immunol. 129: 1256-1261.

Yung, Y.-P., and M.A.S. Moore. 1985. Mast-cell growth factor: Its role in mast-cell differentiation, proliferation, and maturation. Contemp. Top. Mol. Immunol. 10: 147-179.

Yung, Y.-P., S.-Y. Wang, and M.A.S. Moore. 1983. Characterization of mast cell precursors by physical means: Dissociation from T cells and T cell precursors. J. Immunol. 130: 2843-2848.

Yurt, R.W., R.W. Leid, Jr., K.F. Austen, and J.E. Silbert. 1977. Native heparin from rat peritoneal mast cells. J. Biol. Chem. 252: 518-521.

Zucker-Franklin, D. 1980. Ultrastructural evidence for the common origin of human mast cells and basophils. Blood 56: 534-540.

Zucker-Franklin, D., G. Grusky, N. Hirayama, and E. Schnipper. 1981. The presence of mast cell precursors in rat peripheral blood. Blood 58: 544-551.

BIOGRAPHICAL SKETCH

Michael L. Siegel was born on June 15, 1949, in New York City, the younger of two sons of Frances and Seymour Siegel and the brother of Victor (who sometimes claimed to be an only child). After a relatively uneventful childhood and adolescence in Punta Gorda, Florida, Bronx, New York, and Spring Valley, New York, Michael attended Cornell University and managed to achieve a bachelor of science degree in animal science while attending Woodstock, protesting social injustice and the American way, and locking in to age seventeen. Late in his senior year at Cornell, Michael abandoned his lifelong goal of becoming a veterinarian and decided instead to pursue an academic and research career at the University of Florida. His new pursuit was soon postponed, however, as financial problems forced him to withdraw from classes.

Serendipidously, Union Carbide Corporation was looking for someone with Michael's background (so he convinced them), and thus began another era in his life. Michael worked for Union Carbide for five years developing radioimmunoassays, and then accepted an offer from Meloy Laboratories to manage its immunoreagents production unit. Living in Manassas, Virginia, Michael met his wife, Jeremie, and her children, David and Rebecca. In June of

1980, Meloy Laboratories reduced its middle management staff, allowing Michael the opportunity to return to graduate studies at the University of Florida. Following completion of his doctoral studies, Michael will study retroviral oncogenesis and hematopoiesis in the laboratory of Carlo Moscovici, continue his attempts to find renaissance, and, as always, stay seventeen.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward J. Siden, Chairperson
Assistant Professor of Immunology
and Medical Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James B. Flanegan
Associate Professor of Immunology
and Medical Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Carlo Moscovici
Professor of Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

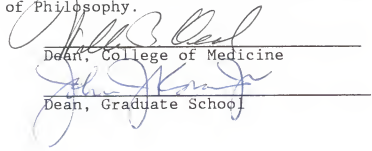
Stephen W. Russell
Professor of Pathology and
Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Roy S. Weiner
Professor of Immunology and
Medical Microbiology

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1986



Dean, College of Medicine

Dean, Graduate School